Supporting Information

Chemoenzymatic Labeling of Proteins for Imaging in Bacterial Cells

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Materials and Methods. Unless otherwise stated, all synthetic reactions were performed using oven-dried glassware and under an atmosphere of argon. Anhydrous solvents (*N,N*-dimethylformamide and dichloromethane) were purchased from Sigma-Aldrich and kept under argon. All other chemicals and reagents for chemical reactions were purchased from commercial vendors (Sigma-Aldrich, Oakwood Chemical, Life Technologies) and used without further purification. Reactions were monitored with thin layer chromatography (EMD/Merck silica gel 60 F254 pre-coated plates) and UV light for visualization, with an acidic mixture of phosphomolybdic acid, cerium ammonium molybdate, or basic aqueous $KMnO_4$ as developing agents. Flash chromatography purifications were carried out using EMD/Merck silica gel 60 (230–400 mesh). ^1H and ^{13}C NMR spectra were measured on either a Bruker Prodigy 400 (400 MHz and 101 MHz, respectively) or a Varian Inova 500 (500 MHz and 126 MHz, respectively), as noted. ^1H and ^{13}C NMR chemical shifts are reported as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS, δ = 0) and calibrated using the residual solvent peak in chloroform (δ 7.26, singlet and δ 77.16, respectively). Data for ^1H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (Hz), and integration. High-resolution mass spectrometry (HRMS) was performed with either a JEOL JMS-600H High Resolution Mass Spectrometer with fast atom bombardment (FAB) at the California Institute of Technology Mass Spectrometry Facility in the Division of Chemistry and Chemical Engineering or with an Agilent 6200 Series TOF with an Agilent G1978A Multimode source in electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), or mixed (MM) ionization mode at the Caltech Beckman Institute Center for Catalysis and Chemical Synthesis. Low-resolution mass spectrometry (LRMS) was performed using an Agilent 1290 UHPLC–MS in positive or negative mode at the Center for Catalysis and Chemical Synthesis.

Lysogeny broth (LB) contained 10 g casein hydrolysate, 5 g yeast extract, and 10 g NaCl per liter. Super optimal broth (SOB) was composed of 20 g casein hydrolysate, 5 g yeast extract, 0.58 g NaCl, 0.19 g KCl, 10 mL 1 M $MgCl_2$, and 10 mL 1M $MgSO_4$ per liter. Super optimal broth with catabolite repression (SOC) was comprised of SOB with the addition of 0.1 mL 2 M glucose to 1 L of SOB medium. Hyper Broth (AthenaES^{TM}, USA) was purchased and prepared according to the manufacturer's instructions. Ampicillin sodium salt (BioPioneer, USA) and kanamycin sulfate (BioPioneer, USA) were used at working concentrations of 200 μg/mL and 35 μg/mL, respectively. Phosphate-buffered saline (PBS) was purchased from Thermo Fisher Scientific (USA) and maintained at pH 7.4. PBST was a solution comprising PBS and 0.1% v/v Tween-20. Q5 Hot Start High-Fidelity DNA Polymerase, restriction endonucleases, Instant Sticky-End Master Mix, Antarctic phosphatase, and Blunt Ligase Master Mix were all purchased from New England Biolabs (USA) and used according to the manufacturer's instructions. DNA constructs were sequenced by Retrogen (USA).

Cells were made chemically competent by using the Mix & Go *E. coli* Transformation Kit (Zymo Research, USA) according to the manufacturer's instructions. Electrocompetent cells were freshly prepared as follows: 8 mL of overnight cultures were washed extensively with 300 mM sucrose on ice. The culture was then concentrated to a final volume of 60 μL cells, after which 20 μL cells were mixed with 50 ng plasmid DNA. Electroporation was carried out using the Gene Pulse XCell (BioRad, USA) according to the recommended manufacturer's protocol. Cells were immediately rescued in 500 μL SOC at 37 °C for 1 h, and plated on LB agar plates carrying the appropriate antibiotic supplements. Plates containing colonies were always used within one week for further experiments.

The plasmid encoding *N*-myristoyltransferase (NMT) and methionyl aminopeptidase (pHV738-NMT-MetAP) was a generous gift from the Kahn laboratory (Emory University, USA). The plasmid confers resistance against kanamycin and contains the p15A origin of replication.[1]

Plasmid construction in pQE80-L. The pQE80-L vector (Qiagen, USA) confers resistance to ampicillin and carries a 6xHis tag following the T5 promoter sequence, *lac* operon and ribosomal binding site (RBS). To remove the 6xHis tag and RBS, pQE80-L was linearized with EcoRI and HindIII. The gene fragment encoding the *N*-terminal nonapeptide sequence from eukaryotic calcineurin B (hCaNB) was 5'– ATG GGT AAC GAA GCG TCT TAC CCG CTG – 3' (encoding the peptide sequence MGNEASYPL for NMT recognition). The gene encoding the chemoreceptor Tar was amplified from genomic DH10B *E. coli* DNA using primers such that the final PCR product read *EcoRI::RBS::hCaNB::BamHI::tar::SacI::6xHis::HindIII*. This PCR product was digested with EcoRI/HindIII and ligated into pQE80-L to yield plasmid pQE80-hCaNB-Tar-6xHis. For the remaining bacterial proteins (CheA, FtsZ, and FtsA) amplification was carried out using genomic DH10B *E. coli* DNA as the template. Primers were designed such that the PCR products read *BamHI::gene of interest::SacI*. The PCR products were digested with BamHI/SacI and ligated into pQE80-hCaNB-Tar-6xHis, which was also digested with BamHI/SacI to yield plasmids pQE80-hCaNB-CheA-6xHis, pQE80-hCaNB-FtsZ-6xHis, and pQE80-hCaNB-FtsA-6xHis. These plasmids were transformed via electroporation into BL21 cells that already harbored plasmid pHV738-NMT-MetAP, generating strains SHH001–004 (Table S3). Colonies were selected against ampicillin and kanamycin. The primers used in this study are summarized in Table S1.

Table S1. Primers used in construction of pQE80-L-based vectors.

Plasmid name	Forward (5' to 3')	Reverse (3' to 5')
pQE80-hCaNB-Tar-6xHis	ATATGAATTCGAGGAGAAA TTAACTATGGGTAACGAAGC GTCTTACCCGCTGGGATCCA TGATTAACCGTATCCGC	TATAAGCTTCTAGTGAT GGTGATGGTGATGGAGC TCAAATGTTTCCCAGTTT GGATC
pQE80-hCaNB-CheA-6xHis	ATATATGGATCCGTGAGCAT GGATATAAGCGATTTTTATC AGACATTTTTTGATGAA	ATATATGAGCTCGGCGG CGGTGTTCGCCAT
pQE80-hCaNB-FtsZ-6xHis	ATATATGGATCCATGTTTGA ACCAATGGAACTT	ATATATGAGCTCATCAG CTTGCTTACGCAG
pQE80-hCaNB-FtsA-6xHis	ATATATGGATCCATGATCAA GGCGACGGAC	ATATATGAGCTCAAACT CTTTTCGCAGCCAACT

Plasmid construction in pBAD24. The pBAD24 vector contains an arabinose-inducible promoter and confers resistance against ampicillin. The gene encoding the methyl aspartate chemoreceptor Tar was amplified from genomic DH10B *E. coli* DNA using primers such that the final PCR product read *EcoRI::hCaNB::SpeI::tar::SacI::6xHis::HindIII*. Both the PCR product and pBAD24 were digested with EcoRI/HindIII and the resulting DNA fragments were ligated to make pBAD24-hCaNB-Tar-6xHis. For the remaining bacterial proteins (CheA, FtsZ, and FtsA, also amplified from genomic DH10B *E. coli* DNA), primers were designed such that the final PCR products read *SpeI::gene of interest::SacI*. The PCR products were digested with SpeI/SacI and ligated into pBAD24-hCaNB-Tar-6xHis, which was also digested with SpeI/SacI

to make the plasmids pBAD24-hCaNB-CheA-6xHis, pBAD24-hCaNB-FtsZ-6xHis, and pBAD24-hCaNB-FtsA-6xHis. To encode the *C*-terminal myc epitope tag and remove the *C*-terminal His tag, phosphorylated primers were used for amplification, with the pBAD24-hCaNB-*gene of interest*-6xHis as the template. (The forward primers for these four constructs are the same and contain the sequence encoding the myc epitope tag, whereas each reverse primer corresponds to a unique bacterial protein.) The template was then digested with DpnI, after which blunt-end ligation was performed to make the plasmids pBAD24-hCaNB-Tar-cmyc, pBAD24-hCaNB-CheA-cmyc, pBAD24-hCaNB-FtsZ-cmyc, and pBAD24-hCaNB-FtsA-cmyc. These plasmids were transformed via electroporation into BL21 cells that already harbored plasmid pHV738-NMT-MetAP, generating strains SHH005–012 (Table S3). Colonies were selected against both ampicillin and kanamycin. The primers used to design these constructs are summarized in Table S2.

Table S2. Primers used in construction of pBAD24-based vectors.

Plasmid name	Forward (5' to 3')	Reverse (3' to 5')
pBAD24-hCaNB-Tar-6xHis	ATATATGAATTCACCATGG GTAACGAAGCGTCTTACCC GCTGACTAGTATGATTAAC CGTATCCGC	TATAAGCTTCTAGTGAT GGTGATGGTGATGGAGC TCAAATGTTTCCCAGTTT GGATC
pBAD24-hCaNB-CheA-6xHis	ATATATACTAGTGTGAGCA TGGATATAAGCGATTTTTA TCAGACATTTTTTGATGAA	ATATATGAGCTCGGCGG CGGTGTTCGCCAT
pBAD24-hCaNB-FtsZ-6xHis	ATATATACTAGTATGTTTG AACCAATGGAACTT	ATATATGAGCTCATCAG CTTGCTTACGCAG
pBAD24-hCaNB-FtsA-6xHis	ATATATACTAGTATGATCA AGGCGACGGAC	ATATATGAGCTCAAACT CTTTTCGCAGCCAACT
pBAD24-hCaNB-Tar-cmyc	GAGCTCGAACAAAAACTTA TTTCTGAAGAAGATCTGTA GAAGCTTGGCTGTTTTGG	AAATGTTTCCCAGTTTG GATC
pBAD24-hCaNB-CheA-cmyc	GAGCTCGAACAAAAACTTA TTTCTGAAGAAGATCTGTA GAAGCTTGGCTGTTTTGG	GGCGGCGGTGTTCGCCA TA
pBAD24-hCaNB-FtsZ-cmyc	GAGCTCGAACAAAAACTTA TTTCTGAAGAAGATCTGTA GAAGCTTGGCTGTTTTGG	ATCAGCTTGCTTACGCA GG
pBAD24-hCaNB-FtsA-cmyc	GAGCTCGAACAAAAACTTA TTTCTGAAGAAGATCTGTA GAAGCTTGGCTGTTTTGG	AAACTCTTTTCGCAGCC AAC

Table S3. *E. coli* strains constructed in this study.

Name	Strain	Genotype
SHH001	BL21	AmpR, KanR pQE80-hCaNB-Tar-6xHis pHV738-NMT-MetAP
SHH002	BL21	AmpR, KanR pQE80-hCaNB-CheA-6xHis pHV738-NMT-MetAP
SHH003	BL21	AmpR, KanR pQE80-hCaNB-FtsZ-6xHis pHV738-NMT-MetAP
SHH004	BL21	AmpR, KanR pQE80-hCaNB-FtsA-6xHis pHV738-NMT-MetAP
SHH005	BL21	AmpR, KanR pBAD24-hCaNB-Tar-6xHis pHV738-NMT-MetAP
SHH006	BL21	AmpR, KanR pBAD24-hCaNB-CheA-6xHis pHV738-NMT-MetAP
SHH007	BL21	AmpR, KanR pBAD24-hCaNB-FtsZ-6xHis pHV738-NMT-MetAP
SHH008	BL21	AmpR, KanR pBAD24-hCaNB-FtsA-6xHis pHV738-NMT-MetAP
SHH009	BL21	AmpR, KanR pBAD24-hCaNB-Tar-myc pHV738-NMT-MetAP
SHH010	BL21	AmpR, KanR pBAD24-hCaNB-CheA-myc pHV738-NMT-MetAP
SHH011	BL21	AmpR, KanR pBAD24-hCaNB-FtsZ-myc pHV738-NMT-MetAP
SHH012	BL21	AmpR, KanR pBAD24-hCaNB-FtsA-myc pHV738-NMT-MetAP

Protein sequences used in this study. The NMT recognition sequence is highlighted in blue. *C*-terminal epitope tags (either 6xHis or myc) are highlighted in red.

pQE80-hCaNB-Tar-6xHis

MGNEASYPLGSMINRIRVVTLLVMVLGVFALLQLISGSLFFSSLHHSQKSFVVSNQLREQQGELTSTWDLMLQT
RINLSRSAVRMMMDSSNQQSNAKVELLDSARKTLAQAATHYKKFKSMAPLPEMVATSRNIDEKYKNYYTALTE
LIDYLDYGNTGAYFAQPTQGMQNAMGEAFAQYALSSEKLYRDIVTDNADDYRFAQWQLAVIALVVVLILLVAWY
GIRRMLLTPLAKIIAHIREIAGGNLANTLTIDGRSEMGDLAQSVSHMQRSLTDTVTHVREGSDAIYAGTREIAAGN
TDLSSRTEQQASALEETAASMEQLTATVKQNADNARQASQLAQSASDTAQHGGKVVDGVVKTMHEIADSSKKI
ADIISVIDGIAFQTNILALNAAVEAARAGEQGRGFAVVAGEVRNLASRSAQAAKEIKALIEDSVSRVDTGSVLVES
AGETMNNIVNAVTRVTDIMGEIASASDEQSRGIDQVALAVSEMDRVTQQNASLVQESAAAAAALEEQASRLTQ
AVSAFRLAASPLTNKPQTPSRPASEQPPAQPRLRIAEQDPNWETFELHHHHHH

pQE80-hCaNB-CheA-6xHis

MGNEASYPLGSVSMDISDFYQTFFDEADELLADMEQHLLVLQPEAPDAEQLNAIFRAAHSIKGGAGTFGFSVL
QETTHLMENLLDEARRGEMQLNTDIINLFLETKDIMQEQLDAYKQSQEPDAASFDYICQALRQLALEAKGETPS
AVTRLSVVAKSEPQDEQSRSQSPRRIILSRLKAGEVDLLEEELGHLTTLTDVVKGADSLSAILPGDIAEDDITAVL
CFVIEADQITFETVEVSPKISTPPVLKLAAEQAPTGRVEREKTTRSNESTSIRVAVEKVDQLINLVGELVITQSMLA
QRSSELDPVNHGDLITSMGQLQRNARDLQESVMSIRMMPMEYVFSRYPRLVRDLAGKLGKQVELTLVGSSTE
LDKSLIERIIDPLTHLVRNSLDHGIELPEKRLAAGKNSVGNLILSAEHQGGNICIEVTDDGAGLNRERILAKAASQG
LTVSENMSDDEVAMLIFAPGFSTAEQVTDVSGRGVGMDVVKRNIQKMGGHVEIQSKQGTGTTIRILLPLTLAILD
GMSVRVADEVFILPLNAVMESLQPREADLHPLAGGERVLEVRGEYLPIVELWKVFNVAGAKTEATQGIVVILQS
GGRRYALLVDQLIGQHQVVVKNLESNYRKVPGISAATILGDGSVALIVDVSALQAINREQRMANTAAELHHHHH
H

pQE80-hCaNB-FtsZ-6xHis

MGNEASYPLGSMFEPMELTNDAVIKVIGVGGGGGNAVEHMVRERIEGVEFFAVNTDAQALRKTAVGQTIQIGS
GITKGLGAGANPEVGRNAADEDRDALRAALEGADMVFIAAGMGGGTGTGAAPVVAEVAKDLGILTVAVVTKPF
NFEGKKRMAFAEQGITELSKHVDSLITIPNDKLLKVLGRGISLLDAFGAANDVLKGAVQGIAELITRPGLMNVDFA
DVRTVMSEMGYAMMGSGVASGEDRAEEAAEMAISSPLLEDIDLSGARGVLVNITAGFDLRLDEFETVGNTIRAF
ASDNATVVIGTSLDPDMNDELRVTVVATGIGMDKRPEITLVTNKQVQQPVMDRYQQHGMAPLTQEQKPVAKV
VNDNAPQTAKEPDYLDIPAFLRKQADELHHHHHH

pQE80-hCaNB-FtsA-6xHis

MGNEASYPLGSMIKATDRKLVVGLEIGTAKVAALVGEVLPDGMVNIIGVGSCPSRGMDKGGVNDLESVVKCVQ
RAIDQAELMADCQISSVYLALSGKHISCQNEIGMVPISEEEVTQEDVENVVHTAKSVRVRDEHRVLHVIPQEYAI
DYQEGIKNPVGLSGVRMQAKVHLITCHNDMAKNIVKAVERCGLKVDQLIFAGLASSYSVLTEDERELGVCVVDI
GGGTMDIAVYTGGALRHTKVIPYAGNVVTSDIAYAFGTPPSDAEAIKVRHGCALGSIVGKDESVEVPSVGGRPP
RSLQRQTLAEVIEPRYTELLNLVNEEILQLQEKLRQQGVKHHLAAGIVLTGGAAQIEGLAACAQRVFHTQVRIGA
PLNITGLTDYAQEPYYSTAVGLLHYGKESHLNGEAEVEKRVTASVGSWIKRLNSWLRKEFELHHHHHH

pBAD24-hCaNB-Tar-cmyc

MGNEASYPLTSMINRIRVVTLLVMVLGVFALLQLISGSLFFSSLHHSQKSFVVSNQLREQQGELTSTWDLMLQT
RINLSRSAVRMMMDSSNQQSNAKVELLDSARKTLAQAATHYKKFKSMAPLPEMVATSRNIDEKYKNYYTALTE
LIDYLDYGNTGAYFAQPTQGMQNAMGEAFAQYALSSEKLYRDIVTDNADDYRFAQWQLAVIALVVVLILLVAWY
GIRRMLLTPLAKIIAHIREIAGGNLANTLTIDGRSEMGDLAQSVSHMQRSLTDTVTHVREGSDAIYAGTREIAAGN
TDLSSRTEQQASALEETAASMEQLTATVKQNADNARQASQLAQSASDTAQHGGKVVDGVVKTMHEIADSSKKI
ADIISVIDGIAFQTNILALNAAVEAARAGEQGRGFAVVAGEVRNLASRSAQAAKEIKALIEDSVSRVDTGSVLVES
AGETMNNIVNAVTRVTDIMGEIASASDEQSRGIDQVALAVSEMDRVTQQNASLVQESAAAAAALEEQASRLTQ
AVSAFRLAASPLTNKPQTPSRPASEQPPAQPRLRIAEQDPNWETFELEQKLISEEDL

pBAD24-hCaNB-CheA-cmyc

MGNEASYPLGSVSMDISDFYQTFFDEADELLADMEQHLLVLQPEAPDAEQLNAIFRAAHSIKGGAGTFGFSVL
QETTHLMENLLDEARRGEMQLNTDIINLFLETKDIMQEQLDAYKQSQEPDAASFDYICQALRQLALEAKGETPS
AVTRLSVVAKSEPQDEQSRSQSPRRIILSRLKAGEVDLLEEELGHLTTLTDVVKGADSLSAILPGDIAEDDITAVL

CFVIEADQITFETVEVSPKISTPPVLKLAAEQAPTGRVEREKTTRSNESTSIRVAVEKVDQLINLVGELVITQSMLA
QRSSELDPVNHGDLITSMGQLQRNARDLQESVMSIRMMPMEYVFSRYPRLVRDLAGKLGKQVELTLVGSSTE
LDKSLIERIIDPLTHLVRNSLDHGIELPEKRLAAGKNSVGNLILSAEHQGGNICIEVTDDGAGLNRERILAKAASQG
LTVSENMSDDEVAMLIFAPGFSTAEQVTDVSGRGVGMDVVKRNIQKMGGHVEIQSKQGTGTTIRILLPLTLAILD
GMSVRVADEVFILPLNAVMESLQPREADLHPLAGGERVLEVRGEYLPIVELWKVFNVAGAKTEATQGIVVILQS
GGRRYALLVDQLIGQHQVVVKNLESNYRKVPGISAATILGDGSVALIVDVSALQAINREQRMANTAAEL**EQKLIS**
EEDL

pBAD24-hCaNB-FtsZ-cmyc
MGNEASYPLGSMFEPMELTNDAVIKVIGVGGGGGNAVEHMVRERIEGVEFFAVNTDAQALRKTAVGQTIQIGS
GITKGLGAGANPEVGRNAADEDRDALRAALEGADMVFIAAGMGGGTGTGAAPVVAEVAKDLGILTVAVVTKPF
NFEGKKRMAFAEQGITELSKHVDSLITIPNDKLLKVLGRGISLLDAFGAANDVLKGAVQGIAELITRPGLMNVDFA
DVRTVMSEMGYAMMGSGVASGEDRAEEAAEMAISSPLLEDIDLSGARGVLVNITAGFDLRLDEFETVGNTIRAF
ASDNATVVIGTSLDPDMNDELRVTVVATGIGMDKRPEITLVTNKQVQQPVMDRYQQHGMAPLTQEQKPVAKV
VNDNAPQTAKEPDYLDIPAFLRKQADEL**EQKLISEEDL**

pBAD24-hCaNB-FtsA-cmyc
MGNEASYPLGSMIKATDRKLVVGLEIGTAKVAALVGEVLPDGMVNIIGVGSCPSRGMDKGGVNDLESVVKCVQ
RAIDQAELMADCQISSVYLALSGKHISCQNEIGMVPISEEEVTQEDVENVVHTAKSVRVRDEHRVLHVIPQEYAI
DYQEGIKNPVGLSGVRMQAKVHLITCHNDMAKNIVKAVERCGLKVDQLIFAGLASSYSVLTEDERELGVCVVDI
GGGTMDIAVYTGGALRHTKVIPYAGNVVTSDIAYAFGTPPSDAEAIKVRHGCALGSIVGKDESVEVPSVGGRPP
RSLQRQTLAEVIEPRYTELLNLVNEEILQLQEKLRQQGVKHHLAAGIVLTGGAAQIEGLAACAQRVFHTQVRIGA
PLNITGLTDYAQEPYYSTAVGLLHYGKESHLNGEAEVEKRVTASVGSWIKRLNSWLRKEFEL**EQKLISEEDL**

Gel analyses. Sodium dodecyl sulfate–poly(acrylamide) gel electrophoresis (SDS–PAGE) was accomplished on a FB3000Q protein gel electrophoresis apparatus from Fisher Scientific (USA) using NuPAGE Novex 4–12% Bis-Tris protein gels (1.0 mm, 10-well) from Thermo Fisher (USA). Protein samples were mixed with an SDS loading buffer consisting of 0.05% w/v bromophenol blue, 0.1 M dithiothreitol (DTT), 10% w/v glycerol, 2% w/v sodium dodecyl sulfate, and 8 M urea in 50 mM Tris, pH 8.0. Samples were heated at 95 °C for 10 min to ensure denaturation and reduction of disulfide bonds. SeeBlue Plus 2 (Thermo Fisher, USA) was used as a molecular weight marker for all SDS–PAGE experiments. Total protein loading was visualized by using InstantBlue (Expedeon, Carlsbad, USA) as a coomassie blue stain, following the manufacturer's instructions.

Protein expression and labeling with ω-azido fatty acids. Individual colonies were used to inoculate LB medium supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin, and cultures were grown overnight at 37 °C with mild agitation (250 rpm). Cultures were diluted 1:50 in 10 mL LB medium (also supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin) and grown until the optical density at 600 nm (OD_{600}) reached 0.5. Protein expression was induced with either 1 mM isopropyl-β-D-1-thiogalactopyrannoside (IPTG) or 0.2% w/v L-(+)-arabinose (for pQE80-L or pBAD24 vectors, respectively). At the time of induction, 500 μM of either **1** or **3** was added to the culture; growth was continued for an additional 4 h (37 °C, 250 rpm). Cultures were harvested, washed twice with PBS, and resuspended in a solution containing 1% w/v sodium dodecyl sulfate (SDS) in Tris pH 8.4. Lysis was accomplished by boiling cells at 90 °C for 5 min, after which the solution was cooled on ice. Benzonase nuclease (Sigma-Aldrich, USA) and protease inhibitor (cOmplete, EDTA-free, Roche, USA) were added to the lysate to degrade chromosomal DNA and to prevent protein proteolysis, respectively. The lysate was allowed to incubate at 37 °C for 1 h, after which the lysates were centrifuged at 13,000 rpm at 4 °C for 30 min. The supernatant was collected, and protein

quantification was performed using the bicinchoninic acid (BCA) assay kit (Thermo Fisher, USA). Lysates were separated into aliquots and stored at -80 °C until further use. Protein expression and labeling with fatty acids were performed in three independent replicate experiments.

Cell viability with compounds 1–3. Individual colonies were used to inoculate LB medium supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin, and cultures were grown overnight at 37 °C with mild agitation (250 rpm). Cultures were diluted to an OD_{600} of 0.1 in LB medium (also supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin). To each culture was added 250 μM **1** or **3**, or 20 μM fluorophore **2**. Cultures were allowed to continue to grow in the presence of each compound, and OD_{600} was monitored every 10 min for 800 min using a Varioskan LUX microplate reader (Thermo Fisher, USA). The OD_{600} of cultures grown without the presence of **1**–**3** were also measured as a control.

Preparation of fatty acid methyl esters (FAMEs) and mass analysis. Individual colonies were used to inoculate LB medium supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin, and cultures were grown overnight at 37 °C with mild agitation (250 rpm). Cultures were diluted 1:50 in 10 mL LB medium (also supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin) and grown until OD_{600} reached 0.5. To each culture was added 250 μM **1** or **3**, or no fatty acid mimic as a control. Growth was continued (37 °C, 250 rpm) until OD_{600} reached approximately 1.0. Cultures were harvested, washed twice with 0.5X PBS, and once with ddH_2O before being lyophilized. Fatty acids were extracted and methylated in a single acidic methylation step.[2] Briefly, freeze-dried cell pellets (approximately 8.0 mg) were resuspended in a 20: 1 v/v methanol: acetyl chloride solution and heated at 100 °C for 10 min. The solutions were allowed to cool to room temperature, after which 1 mL hexane and 1 mL water were added, and FAMEs were extracted twice with hexane (2 x 1 mL). The organic layers were combined and concentrated to a final volume of 200 μL for mass analysis. GC–MS analysis of FAMEs was accomplished using a ThermoFinnigan Trace GC equipped with a HP-5MS column (30 m x 0.250 mm x 0.25 μm), with the column effluent split between a flame ionization detector (FID) and ThermoFinnigan DSQ mass spectrometer. The GC oven was held at 90 °C for 1 min, ramped at 3 °C / min to 140 °C, ramped at 3 °C / min to 250 °C, and finally ramped at 20 °C / min to a final temperature of 310 °C. Known FAMEs present in *E. coli* were identified using the NIST MS Search 2.0 program and quantified against an internal standard (methyl behenate). GC traces of FAME extracts of cells not treated with fatty acids were compared with GC traces of FAME extracts of cells treated with fatty acids to identify new peaks.

Lipid extraction from cells grown with 1 or 3 and mass analysis. Individual colonies were used to inoculate LB medium supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin, and cultures were grown overnight at 37 °C with mild agitation (250 rpm). Cultures were diluted 1:50 in 10 mL LB medium (also supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin) and grown until OD_{600} reached 0.5. To each culture was added 250 μM **1** or **3**, or no fatty acid mimic as a control. Growth was continued (37 °C, 250 rpm) until OD_{600} reached approximately 1.0. Cultures were harvested and washed twice with 0.5X PBS. Lipid extraction was performed following literature protocols.[3] Briefly, cell pellets were resuspended in 120 μL 0.1% wt/v ammonium acetate (dissolved in ddH_2O). To the solution was added 300 μL methanol and 1 mL methyl-*tert*-butyl ether (MTBE). Solutions were briefly vortexed and

then sonicated for 1 hour at room temperature using a sonicator bath. 8 μg of internal standard (di17:0 phosphatidylcholine, phosphatidylethanolamine, and phosphatidylglycerol) (Avanti Polar Lipids, Alabaster, USA) was added at this time. Internal standards were prepared as 0.1 g/L solutions in methanol/dichloromethane (9:1) Lipids were extracted by addition of 260 μL of water, forming a biphasic solution, with lipids in the top (MTBE/methanol) layer. The MTBE/methanol layer was separated from the water layer and dried under a gentle stream of nitrogen. Dried samples were dissolved in 1 mL 9:1 methanol/dichloromethane for analysis using LC–MS. Fatty acids **1** and **3** were also dissolved to a final concentration of 10 μM in 9:1 methanol/dichloromethane as standards for mass spectrometry. Samples were submitted to the Caltech Environmental Analysis Center of the Beckman Institute. Mass spectrometry (UPLC–MS) analysis was accomplished using an Acquity I-Class UPLC coupled to a Xevo G2-S TOF mass spectrometer (Waters, USA). Lipid samples (injection volume, 5 μL) were separated on an Acquity UPLC CSH C18 column (2.1 μm x 100 mm, 1.7 μm, Waters, USA). UPLC–TOF–MS^E data were collected in positive and negative mode using electrospray ionization (ESI). Indiscriminate fragmentation of all parent ions was performed with the capillary voltage set to 2.0 KV, the cone voltage set to 30 V, and the acquisition range set for data collection between 100 to 2000 m/z. MS^E data consists of two channels acquired simultaneously. Extracted ion chromatograms were performed using m/z searches based on fragment ions with a mass window of 10 mDa. Lipids were identified from their fragmentation patterns in both negative and positive mode.

Fluorescence labeling in cell lysates. To 100 μg protein in cell lysate diluted to 250 μL with PBS, was added iodoacetamide to a final concentration of 1 mM. Lysates were incubated at 37 °C for 1 h in the dark. Then, **2** was added to each solution to a final concentration of 2 μM. Reactions were performed at room temperature for 10 min in the dark. Proteins were then precipitated by addition of chloroform and methanol. Pellets were resuspended in SDS loading buffer. Samples were boiled for 10 min at 95 °C, after which 20 μg lysate were subjected to SDS–PAGE. Protein gels were washed with a solution containing 10% acetic acid, 20% methanol, and 70% water, with mild agitation (2 x 30 min). Fluorescence labeling was visualized using a Typhoon Trio (GE Healthcare, USA) with excitation at 488 nm, the photomultiplier tube (PMT) voltage set to 400 V, and emission monitored using a 520 nm band pass filter. Protein bands corresponding to the fluorescently labeled protein of interest were quantified using ImageQuant TL (GE Healthcare, USA), and normalized against the intensity of that particular band in the coomassie lane. Fluorescence labeling was quantified for each biological replicate. Plots representing the average fluorescence intensities from independent experiments were prepared using IGOR Pro (Wavemetrics, Oregon, USA).

Immunoblotting. Protein lysates were separated by SDS–PAGE and transferred to a 0.2 μm nitrocellulose membrane using an iBlot 2 gel transfer apparatus from Life Technologies (USA), following the manufacturer's protocol. Membranes were blocked with 5% w/v non-fat dry milk in PBST at room temperature for 1 h, and washed three times with PBST before incubating with either 1:5000 PentaHis–Alexa Fluor 647 (Qiagen, USA), or 1:2000 c–myc Alexa Fluor 647 conjugate (Cell Signaling Technologies, USA) at 4 °C overnight. Membranes (covered from light) were then washed five times with PBST at room temperature (10 min each wash). Fluorescence was visualized using a Typhoon Trio (GE Healthcare, USA) with excitation at 633 nm, the photomultiplier tube (PMT) voltage set to 300 V, and emission monitored using a 670 nm band pass filter.

Expression, purification and mass spectrometry of Tar. An overnight culture of *E. coli* strain SHH001 bearing plasmids pQE80-hCaNB-Tar-6xHis and pHV738-NMT-MetAP was diluted 1:50 in 100 mL Hyper Broth supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin and grown at 37 °C with mild agitation (180 rpm). When OD_{600} reached approximately 0.5, the culture was cooled on ice for 20 min, after which 500 μM of either **1** or **3** and 0.025 mM IPTG were added to induce protein expression and to initiate *N*-terminal labeling. Cells were grown for an additional 20 h at reduced temperature and agitation speed (20 °C, 140 rpm), harvested, and stored at -80 °C until use. Cell pellets were thawed and resuspended in a solution (4 mL/g cell mass) containing 50 mM NaH_2PO_4, 300 mM NaCl, 1 mg/mL lysozyme, pH 8.0, and incubated on ice for 30 min. Lysis was achieved using a Q500 Sonicator (QSonica, USA), operating at a power of 500 watts and frequency of 20 kHz, with 5 s on and 25 s off bursts at 40% amplitude (1 min total for on cycle). The lysates were centrifuged at 13,000 rpm at 4 °C for 30 min, and the supernatant was discarded. To the insoluble fraction was added a solution (10 mL) containing 100 mM NaH_2PO_4, 10 mM Tris, 8 M urea, 1% w/v n-dodecyl β-D-maltoside (DDM), pH 8.0, and incubated at 4 °C with mild agitation for 30 min. Lysates were centrifuged at 13,000 rpm at 4 °C for 30 min, after which the protein was purified from lysate using Ni–NTA agarose (Qiagen, USA) under denaturing conditions. Briefly, 10 mL lysate was incubated with 250 μL Ni–NTA agarose at room temperature for 30 min and loaded onto the column. The column was washed with 10 column volumes of Buffer A (100 mM NaH_2PO_4, 10 mM Tris, 8 M urea, 0.2% w/v DDM, pH 8.0) and 10 column volumes of Buffer B (100 mM NaH_2PO_4, 10 mM Tris, 8 M urea, 0.2% w/v DDM, pH 6.3). The protein was eluted in 4 column volumes of Buffer C (100 mM NaH_2PO_4, 10 mM Tris, 8 M urea, 0.2% w/v DDM, pH 5.9) and 5 column volumes of Buffer D (100 mM NaH_2PO_4, 10 mM Tris, 8 M urea, 0.2% w/v DDM, pH 4.5). Fractions containing purified Tar were pooled and dialyzed against 25 mM Tris, 50 mM NaCl, 0.2% w/v DDM, pH 8.0 for 24 h. The protein was diluted in a solution of 0.2% v/v formic acid in 95% ddH_2O and 5% acetonitrile to a final concentration of 20 μM. Protein samples were submitted for LC–MS analysis using an Agilent 1100 MSD equipped with a quadrupole detector (Agilent Technologies, USA) at the Proteome Exploration Laboratory of the Beckman Institute at Caltech.

Expression, purification, and mass spectrometry of CheA. An overnight culture of *E. coli* strain SHH002 bearing plasmids pQE80-hCaNB-CheA-6xHis and pHV738-NMT-MetAP was diluted 1:50 in 100 mL Hyper Broth supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin and grown at 37 °C with mild agitation (180 rpm). When OD_{600} reached approximately 0.5, the culture was cooled on ice for 20 min, after which 500 μM of either **1** or **3** and 0.025 mM IPTG were added to induce protein expression and to initiate *N*-terminal labeling. Cells were grown for an additional 20 h at reduced temperature and agitation speed (20 °C, 140 rpm), harvested, and stored at -80 °C until use. Pellets were thawed and resuspended in a solution (4 mL/g cell mass) containing 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, 1 mg/mL lysozyme, pH 8.0, and incubated on ice for 30 min. Lysis was achieved using a Q500 Sonicator (QSonica, USA), operating at a power of 500 watts and frequency of 20 kHz, with 5 s on and 25 s off bursts at 40% amplitude (1 min total for on cycle). The lysates were centrifuged at 13,000 rpm at 4 °C for 30 min. Clarified lysates were loaded onto Ni–NTA columns (1 mL size, HP resin, GE Healthcare) using an ÄKTA purifier FPLC system (GE Healthcare, USA). CheA was eluted from the column using a linear gradient from 100% Buffer A' (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0), 0% Buffer B' (50 mM NaH_2PO_4, 300 mM NaCl, 500 mM imidazole, pH 8.0) to 100% Buffer B' over 20 column volumes. Fractions

containing CheA were pooled and buffer exchanged into an anion exchange buffer (25 mM Tris, 10 mM NaCl, pH 8.0) and loaded onto an anion exchange Q-Sepharose column (HiTrapTM Q HP, GE Healthcare) using an ÄKTA purifier FPLC system (GE Healthcare, USA). CheA was eluted from the Q-column by running a linear gradient from 10 mM NaCl to 1 M NaCl over 20 column volumes. The protein was diluted in a solution of 0.2% v/v formic acid in 95% ddH_2O and 5% acetonitrile to a final concentration of 20 μM. Protein samples were submitted for LC–MS analysis using an Agilent 1100 MSD equipped with a quadrupole detector (Agilent Technologies, USA) at the Proteome Exploration Laboratory of the Beckman Institute at Caltech.

Expression, purification, and mass spectrometry of FtsZ. An overnight culture of *E. coli* strain SHH003 bearing plasmids pQE80-hCaNB-FtsZ-6xHis and pHV738-NMT-MetAP was diluted 1:50 in 10 mL LB medium supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin and grown at 37 °C with mild agitation (250 rpm). When OD_{600} reached approximately 0.5, 500 μM of either **1** or **3** and 1 mM IPTG were added to the culture to induce protein expression and to initiate *N*-terminal labeling. Cells were grown for an additional 4 h (37 °C, 250 rpm) and harvested. Cell pellets were resuspended in a solution containing 50 mM NaH_2PO_4, 300 mM NaCl, 5 mM imidazole, 1 mg/mL lysozyme, and incubated on ice for 30 min. Lysis was achieved using a Q500 Sonicator (QSonica, USA), operating at a power of 500 watts and frequency of 20 kHz, with 5 s on and 25 s off bursts at 30% amplitude (1 min total for on cycle). The lysates were centrifuged at 13,000 rpm at 4 °C for 30 min, after which the protein was purified from lysate using Ni–NTA agarose spin columns (Qiagen, USA) according to the manufacturer's instructions. The purified protein was eluted in a buffer solution containing 50 mM NaH_2PO_4, 300 mM NaCl, and 500 mM imidazole. The purified protein was further concentrated using 30 kDa MWCO spin filters (Millipore, USA) and the buffer was exchanged into a solution containing 50 mM NH_4HCO_3. The protein was diluted in a solution of 0.2% v/v formic acid in 95% ddH_2O and 5% acetonitrile to a final concentration of 20 μM. ESI–TOF mass spectrometry was performed using an LCT Premier XE mass spectrometer (Waters, USA) at the Mass Spectrometry Facility in the Division of Chemistry and Chemical Engineering at Caltech. Mass spectra were deconvoluted using MassLynx V 4.1 (Waters, USA) and spectra were plotted using IGOR Pro (Wavemetrics, Oregon, USA).

Expression, purification, and mass spectrometry of FtsA. An overnight culture of *E. coli* strain SHH004 bearing plasmids pQE80-hCaNB-FtsA-6xHis and pHV738-NMT-MetAP was diluted 1:50 in 100 mL Hyper Broth supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin and grown at 37 °C with mild agitation (180 rpm). When OD_{600} reached approximately 0.5, the culture was cooled on ice for 20 min, after which 500 μM of either **1** or **3** and 0.025 mM IPTG were added to induce protein expression and to initiate *N*-terminal labeling. Cells were grown for an additional 20 h at reduced temperature and agitation speed (20 °C, 140 rpm), harvested, and stored at -80 °C until use. Cell pellets were resuspended in a solution (4 mL/g cell mass) containing 100 mM NaH_2PO_4, 10 mM Tris, 8 M urea, pH 8.0, and were lysed at 4 °C with mild agitation for 60 min. Lysates were clarified with centrifugation (13,000 rpm, 4 °C, 30 min). Clarified lysate was incubated with 250 μL Ni–NTA agarose (Qiagen, USA) at room temperature for 30 min before being loaded onto the column. The column was washed with 10 column volumes of 100 mM NaH_2PO_4, 10 mM Tris, 8 M urea, pH 8.0 and 15 column volumes of 100 mM NaH_2PO_4, 10 mM Tris, 8 M urea, pH 6.3. FtsA was eluted from the column with 5 column volumes of 100 mM NaH_2PO_4, 10 mM Tris, 8 M urea, pH 5.9, and 5 column volumes of

100 mM NaH_2PO_4, 10 mM Tris, 8 M urea, pH 4.5. Fractions containing FtsA were pooled and dialyzed against 25 mM Tris, 50 mM NaCl, pH 8.0 overnight. The protein was diluted in a solution of 0.2% v/v formic acid in 95% ddH_2O and 5% acetonitrile to a final concentration of 20 μM. Protein samples were submitted for LC–MS analysis using an Agilent 1100 MSD equipped with a quadrupole detector (Agilent Technologies, USA) at the Proteome Exploration Laboratory of the Beckman Institute at Caltech.

Mass calibration for the Agilent 1100 MSD was performed with an internal standard of myoglobin in positive mode. Mass calibration for the LCT Premier XE mass spectrometer was performed with an internal standard consisting of sodium iodide clusters.

Calculated and observed masses are summarized in Tables S4–S7. Protein masses were calculated using http://www.expasy.org. Masses for proteins modified with **1** or **3** take into account for the following: the loss of the starting Met, the addition of the fatty acid, and the loss of a water molecule due to amide bond formation between the fatty acid and the Gly residue. In cells that were not labeled with fatty acids, we observed that proteins of interest were labeled with endogenous myristic acid.[4] Additionally, we observed the mass addition of a phosphoryl group on unmodified CheA. CheA has been reported to be autophosphorylated in bacterial cells.[5]

Table S4. Calculated and observed masses for modification of Tar.

Calculated mass (Da)	Observed mass (Da)	+/- (Da)	Notes
61984.8	61984.9	4.5	Unmodified Tar (–Met)
62208.0	Not observed		Tar modified with **1**
62229.8	62226.9	4.1	Tar modified with **1** (sodium adduct)
62210.0	62210.2	4.6	Tar modified with **3**

Table S5. Calculated and observed masses for modification of CheA.

Calculated mass (Da)	Observed mass (Da)	+/- (Da)	Notes
73391.5	73391.2	5.2	Unmodified CheA (–Met)
73470.5	73471.8	4.6	CheA modified by phosphorylation
73614.7	Not observed		CheA modified with **1**
73636.7	73635.9	5.1	CheA modified with **1** (sodium adduct)
73616.7	Not observed	4.9	CheA modified with **3**
73638.7	73638.7	5.1	CheA modified with **3** (sodium adduct)

Table S6. Calculated and observed masses for modification of FtsZ.

Calculated mass (Da)	Observed mass (Da)	+/- (Da)	Notes
42365.1	42364.8	1.1	Unmodified FtsZ (–Met)
42575.5	42575.1	1.0	FtsZ modified with endogenous myristic acid
42588.3	42588.2	1.0	FtsZ modified with **1**
42562.3	42562.2	1.0	FtsZ modified with **1** (reduction of azide to amine)
42590.3	42590.1	1.2	FtsZ modified with **3**

Table S7. Calculated and observed masses for modification of FtsA.

Calculated mass (Da)	Observed mass (Da)	+/- (Da)	Notes
47371.1	Not observed		Unmodified FtsA (–Met)
47581.5	47582.6	3.1	FtsA modified with endogenous myristic acid
47594.3	47594.6	3.5	FtsA modified with **1**
47596.3	47595.8	2.4	FtsA modified with **3**

Labeling bacterial proteins with ω-azido fatty acids in fixed cells. Overnight cultures were diluted 1:50 in LB medium supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin and grown at 37°C with mild agitation (250 rpm). Cells (harboring pBAD24-derived and pHV738-NMT-MetAP plasmids) expressing Tar or CheA were grown to an OD_{600} of 0.5, and cells expressing FtsZ and FtsA were grown to an OD_{600} of 0.9, after which 0.2% w/v arabinose and 250 μM **1** were added to the cultures. Cells were allowed to grow for an additional 2 h, after which aliquots of cells were concentrated to an OD_{600} of approximately 2. Cells were fixed by addition of 4% paraformaldehyde in PBS for 10 min at room temperature, and permeabilized with 0.3% v/v Triton X-100 for 15 min at room temperature. Proteins were alkylated by addition of 100 mM iodoacetamide at room temperature for 1 h in the dark. *N*-terminal fluorescence labeling was carried out by incubating the cells with 20 μM **2** at room temperature for 30 min. Cells were rinsed four times with PBS and blocked with 3% w/v BSA in PBS at room temperature for 1 h, after which c-myc Alexa Fluor 647 antibody (Cell Signaling Technologies, USA) was added to the solution at a 1:100 dilution. Cells were allowed to incubate with the antibody solution overnight at 4 °C, and washed five times with PBS the following day. Cells were then mounted on 5% w/v agarose (in PBS) slides for imaging.

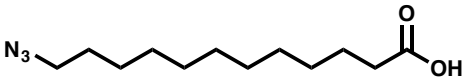
Labeling bacterial proteins with ω-azido fatty acids in live cells. Overnight cultures (harboring modified pBAD24 and pH738-NMT-MetAP plasmids) were diluted 1:50 in LB medium supplemented with 200 μg/mL ampicillin and 35 μg/mL kanamycin and grown at 37°C with mild agitation (250 rpm). Cells expressing Tar or CheA were grown to an OD_{600} of 0.5, and cells expressing FtsZ and FtsA were grown to an OD_{600} of 0.9, after which 0.2% w/v arabinose and 250 μM **1** or **3** were added to the cultures. Cells were allowed to grow for an additional 2 h, after which aliquots of cells were concentrated to an OD_{600} of approximately 2. Cells were rinsed three times with PBS, after which **2** was added to a 100 μL aliquot of cell suspension to a final concentration of 20 μM. The cells were incubated with **2** at 37°C for 30 min, after which the solution was washed three times with PBS to remove excess fluorophore. Cells were then mounted onto a 5% w/v agarose slide in PBS for imaging.

Fluorescence microscopy. Protein localization in cells was detected using an inverted Zeiss LSM 5 Exciter laser scanning confocal microscope at the Biological Imaging Facility of the Beckman Institute at Caltech. A 488 nm laser line (25 mW argon laser, 1–2 %) and 505–550 nm band pass filter were used in the detection of proteins labeled by **2**. Excitation at 633 nm (5 mW neon laser, 1–5 %) and emission at 650 nm (long pass) were used for immunofluorescence detection. Images were taken as 15 Z-stacks, with 0.2 μm per stack. ImageJ (NIH) was used to sum each Z-stack to form the projections represented in the figures. Cell Profiler was used to quantify the integrated fluorescence per cell for approximately 100–150 cells per sample.[6]

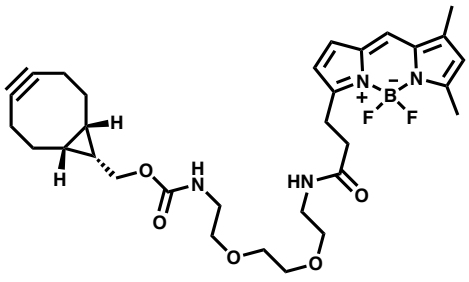
Discussion on fatty acid probes 1 and 3. Gordon and coworkers previously showed that heteroatom-containing myristic acid analogues are well-tolerated by NMT, and that replacement of a methylene group by an ether oxygen atom is roughly equivalent to deletion of four methylene groups in terms of reduction in the hydrophobic character of the acid (Ref. 7). Estimated logP values for **1** and **3** were calculated using Molinspiration Chemoinformatics (www.molinspiration.com), resulting in logP values of 4.84 and 2.86, respectively. The software uses group contribution values to determine estimated partition coefficients. For a discussion on group contributions, see Ref. 8.

Discussion on fatty acid pool and intact lipids in *E. coli*. We analyzed the free fatty acid pool of bacterial cells that were treated with the azide-containing fatty acids (Fig. S9) by GC–MS. Cells treated with **1** showed a peak in the GC trace that matched that of the methyl ester of **1**; otherwise, the GC trace was identical to that of untreated cells. We looked at the intact phospholipids by LC–MS (Fig. S10–12). In cells treated with **1**, we observed a single new peak that was not present in untreated cells. On the basis of extracted ions in negative mode, we conclude that the phospholipid contained palmitic acid and **1**. Fragmentation in positive mode showed a m/z loss of 141 Da, corresponding to a phosphorylethanolamine molecule. This would suggest that *E. coli* incorporates **1** into a PE molecule. Treatment of cells with **3** did not significantly alter the intact lipid profile (Fig. S26).

Synthetic procedures

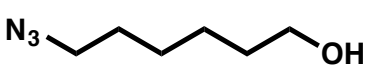


12-azidododecanoic acid (**1**). To a flamed-dried 100 mL round bottom flask, was added 20 mL DMF, 419.1 mg sodium azide (6.45 mmol, 1.80 equiv), and 53.7 mg sodium iodide (0.36 mmol, 0.1 equiv). The mixture was stirred to form a heterogeneous suspension, after which 12-bromododecanoic acid (1.0 g, 3.58 mmol, 1.0 equiv) was added. The reaction mixture was allowed to stir at room temperature for 24 h, after which 20 mL ethyl acetate was added, and the solution was cooled on ice. To the resultant solution, 1 M HCl was added dropwise until the suspension became clear. The reaction mixture was extracted twice with ethyl acetate (2 x 20 mL). The organic layer was washed three times with saturated LiCl (3 x 20 mL), dried over $MgSO_4$, and concentrated in vacuo, resulting in a slightly yellow solid. The crude product was washed extensively with ice-cold ether and ice-cold water before being filtered. The white solid was collected and dried overnight under high vacuum to afford 785.6 mg of **1** as a brilliant, white solid (91%). (400 MHz, $CDCl_3$): δ 3.25 (t, 2H, J = 6.8 Hz), 2.35 (t, 2H, J = 7.6 Hz), 1.58 – 1.65 (m, 4H), 1.30 – 1.36 (m, 6H), 1.25 – 1.30 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 179.67, 77.36, 51.63, 34.08, 29.58, 29.50, 29.35, 29.28, 29.17, 28.98, 26.85, 24.80. HRMS (FAB) calculated for $C_{12}H_{24}N_3O_2$ ($[M+H]^+$) 242.1868, found 242.1853.

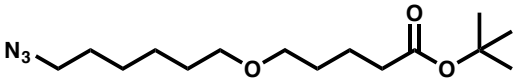


BCN–BODIPY conjugate (**2**). An oven-dried 20 mL scintillation vial was charged with 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionic acid (5 mg, 17 μmol, 1.0 equiv), *O*-(6-chlorobenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HCTU) (7 mg, 17 μmol, 1.0 equiv), and *N*-(1*R*,8*S*,9*s*)-bicyclo[6.1.0]non-4-yn-9-ylmethyloxycarbonyl]-1,8-diamino-3,6-dioxaoctane (10 mg, 31 μmol, 1.8 equiv). *N,N*-dimethylformamide (2 mL) was then added to the vial. To the solution, *N,N*-diisopropylethylamine (DIPEA) (6.7 μL, 39 μmol, 2.3 equiv) was added, and the reaction mixture was allowed to stir at room temperature for 4 h, after which the solvent was removed *in vacuo*. The residue was taken up in 10 mL ethyl acetate and extracted twice with 1 M HCl (2 x 20 mL), washed once with deionized water (10 mL), and washed three times with saturated LiCl (3 x 30 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give a lustrous brown oil as the crude product. Flash chromatography (1–2% methanol in dichloromethane) afforded 9.2 mg of **2** as a brilliant, red solid (90%). ^{1}H NMR (400 MHz,

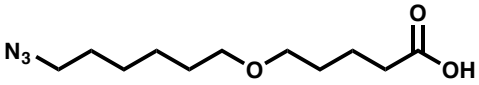
$CDCl_3$): δ, 7.08 (s, 1H), 6.89 (d, 1H, J = 4.0 Hz), 6.30 (d, 1H, J = 4.0 Hz), 6.12 (br s, 1H), 5.22 (br s, 1H), 4.14 (br s, 1H), 4.12 (br s, 1H), 3.57 (br s, 1H), 3.54 – 3.50 (m, 4H), 3.46 – 3.42 (m, 2H), 3.37 – 3.33 (m, 2H), 3.30 – 3.25 (m, 2H), 2.63 (t, 2H, J = 8.0 Hz), 2.56 (s, 3H), 2.25 (s, 3H), 2.23 – 2.21 (m, 2H), 1.61 – 1.50 (m, 7H), 1.33 (t, 2H, J = 8.8 Hz), 0.91 (t, 2H, J = 9.6 Hz). ^{13}C NMR (101 MHz, $CDCl_3$): δ, 171.89, 160.27, 157.71, 156.90, 143.92, 135.20, 133.49, 128.37, 123.88, 120.52, 117.63, 98.96, 70.39, 70.33, 70.24, 70.04, 62.90, 40.88, 39.39, 36.11, 29.16, 24.99, 21.57, 20.22, 17.90, 15.09, 11.48. HRMS (FAB) calculated for $C_{31}H_{42}O_5N_4BF_2$ ($[M+H]^+$) 599.3216, found 599.3219.



6-azidohexan-1-ol (**S1**). To a flamed-dried 100 mL round bottom flask was added sodium azide (968.9 mg, 14.9 mmol, 1.8 equiv), sodium iodide (123.4 mg, 0.83 mmol, 0.1 equiv), and 10 mL DMF to form a heterogeneous suspension. To this mixture was added 6-bromohexan-1-ol (1.5 g, 8.28 mmol, 1.0 equiv) dropwise over the course of 2 min. The reaction mixture was allowed to stir at room temperature for 18 h, after which the solution was diluted with 30 mL ethyl acetate and cooled on ice. To the solution, 10 mL 1 M HCl was slowly added to quench the reaction. The solution was extracted twice with 1 M HCl (2 x 10 mL), twice with deionized water (2 x 10 mL), twice with saturated LiCl (2 x 10 mL), dried over $MgSO_4$, and concentrated *in vacuo*. Flash chromatography (10% ethyl acetate: 90% hexanes) afforded 1.07 g of **S1** as a colorless oil (90%). ^{1}H NMR (500 MHz, $CDCl_3$): δ, 3.66 (t, 2H, J = 6.5 Hz), 3.28 (t, 2H, J = 7.0 Hz), 1.61 (m, 4H), 1.41 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ, 62.93, 51.51, 32.69, 28.95, 26.66, 25.47. LRMS (ESI) calculated for $C_6H_{14}N_3O$ ($[M+H]^+$) 144, found 144.



tert-butyl 5-((6-azidohexyl)oxyl)pentanoate (**S2**). A flame-dried 50 mL flask was charged with **S1** (276 mg, 1.93 mmol, 1.1 equiv), followed by anhydrous DMF (10 mL), and cooled to 0°C on ice. To the solution was slowly added NaH (84.1 mg 60% wt dispersion in mineral oil, 2.10 mmol, 1.2 equiv), and the resulting suspension was allowed to stir on ice for 0.5 h. The solution was warmed to room temperature and allowed to stir for an additional 0.5 h, after which the reaction mixture was cooled on ice. To the reaction mixture was added *tert*-butyl 5-bromopentanoate (415.5 mg, 1.75 mmol, 1.0 equiv) dropwise over the course of 10 min. The reaction mixture was allowed to warm to room temperature and stirred for an additional 5 h, after which the solution was cooled on ice and quenched by addition of aqueous, saturated LiCl (20 mL). The solution was extracted twice with ethyl acetate, dried over $MgSO_4$, and concentrated in vacuo. The residue was suspended in dichloromethane and purified by flash chromatography (1–5% ethyl acetate in hexanes) to afford 146.7 mg of **S2** as a colorless oil (28%). ^{1}H NMR (500 MHz, $CDCl_3$): δ, 3.40 (t, 2H, J = 6.0 Hz), 3.39 (t, 2H, J = 6.5 Hz), 2.23 (t, 2H, J = 7.0 Hz), 1.62 (m, 4H), 1.59 (m, 4H), 1.43 (s, 9H), 1.38 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ, 173.15, 80.16, 70.83, 70.58 51.53, 35.44, 29.74, 29.27, 28.94, 28.25, 26.71, 25.93, 21.99. HRMS (FAB) calculated for $C_{15}H_{30}N_3O_3$ ($[M+H]^+$) 300.2287, found 300.2283.



5-((6-azidohexyl)oxy)pentanoic acid (**3**). A 20 mL scintillation vial was charged with **S2** (140 mg, 0.47 mmol, 1 equiv) and 2 mL dichloromethane. The reaction mixture was allowed to stir to form a homogenous solution, after which 2 mL trifluoroacetic acid was added dropwise over the course of 5 min. The reaction mixture continued to stir at room

temperature for 1 h, after which the trifluoroacetic acid was evaporated using a gentle stream of nitrogen. Remaining trifluoroacetic acid was removed by azeotropic evaporation with toluene (5 x 5 mL) under reduced pressure to afford **3** as a faint yellow oil (quantitative). The material was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ, 3.43 (t, 2H, J = 6.0 Hz), 3.40 (t, 2H, J = 6.0 Hz), 3.26 (t, 2H, J = 7.0 Hz), 2.39 (t, 2H, J = 7.0 Hz), 1.72 (m, 2H), 1.61 (m, 6H), 1.38 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ, 179.08, 70.92, 70.46, 51.53, 33.81, 29.68, 29.09, 28.93, 26.70, 25.92, 21.72. HRMS (MM) calculated for $C_{11}H_{20}N_3O_3$ ($[M-H]^-$) 242.1510, found 242.1507.

Supplementary Figures

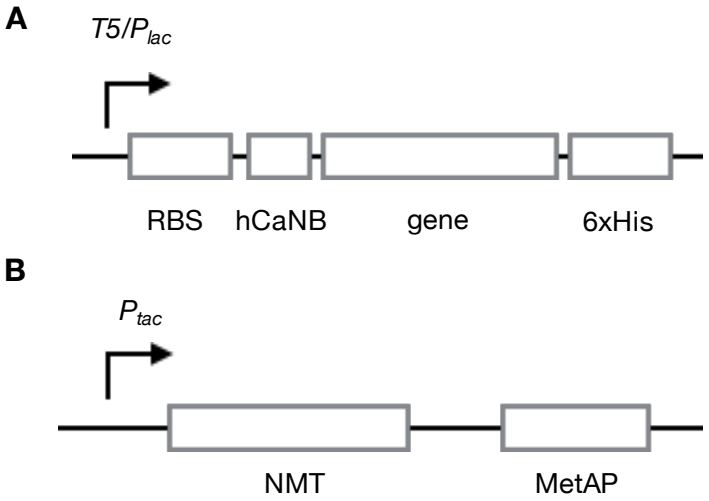


Figure S1. Schematic of vector construction in pQE80-L and pHV738-NMT-MetAP plasmids. (A) The gene encoding the protein of interest is placed under control of the bacteriophage T5 promoter in the pQE80-L vector. The protein is outfitted with the *N*-terminal nonapeptide sequence from calcineurin B (hCaNB) for NMT recognition and a *C*-terminal hexahistidine tag for purification. RBS = ribosome binding site (B) Simplified vector schematic for pHV738-NMT-MetAP (from Ref. 1). Expression of NMT is placed under control of the Ptac promoter whereas methionyl aminopeptidase (MetAP) expression is placed under control of its own promoter. The plasmid harboring the genes encoding NMT and MetAP contains a p15A origin of replication.

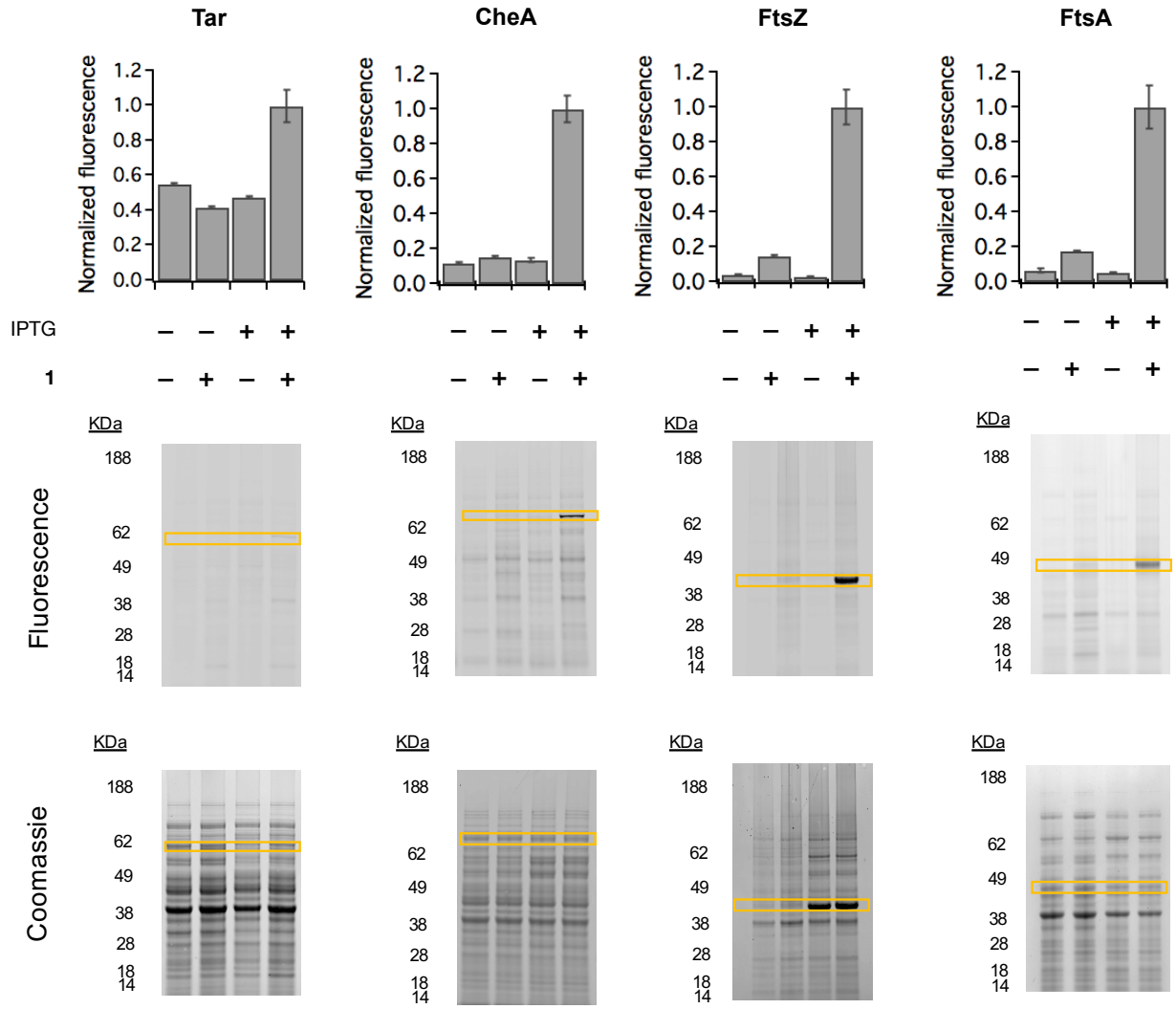


Figure S2. Fluorescence emission from labeled proteins expressed from pQE80-L plasmids. Crude lysates from cells (labeled with **1**) expressing NMT and one of the four bacterial proteins (Tar, CheA, FtsZ, or FtsA) from pQE80-L plasmids were treated with **2** and separated using SDS–PAGE. Fluorescence measurements (from gels corresponding to Figure 2) were normalized to the band corresponding to the labeled protein in the coomassie lane. Error bars denote standard deviations from three independent experiments. Shown below the bar graphs are the fluorescence and coomassie gels in inverted grayscale. The regions boxed in yellow denote the band regions which were used in quantifying fluorescence emission. Fluorescence emission was normalized against the intensity from the associated coomassie gels.

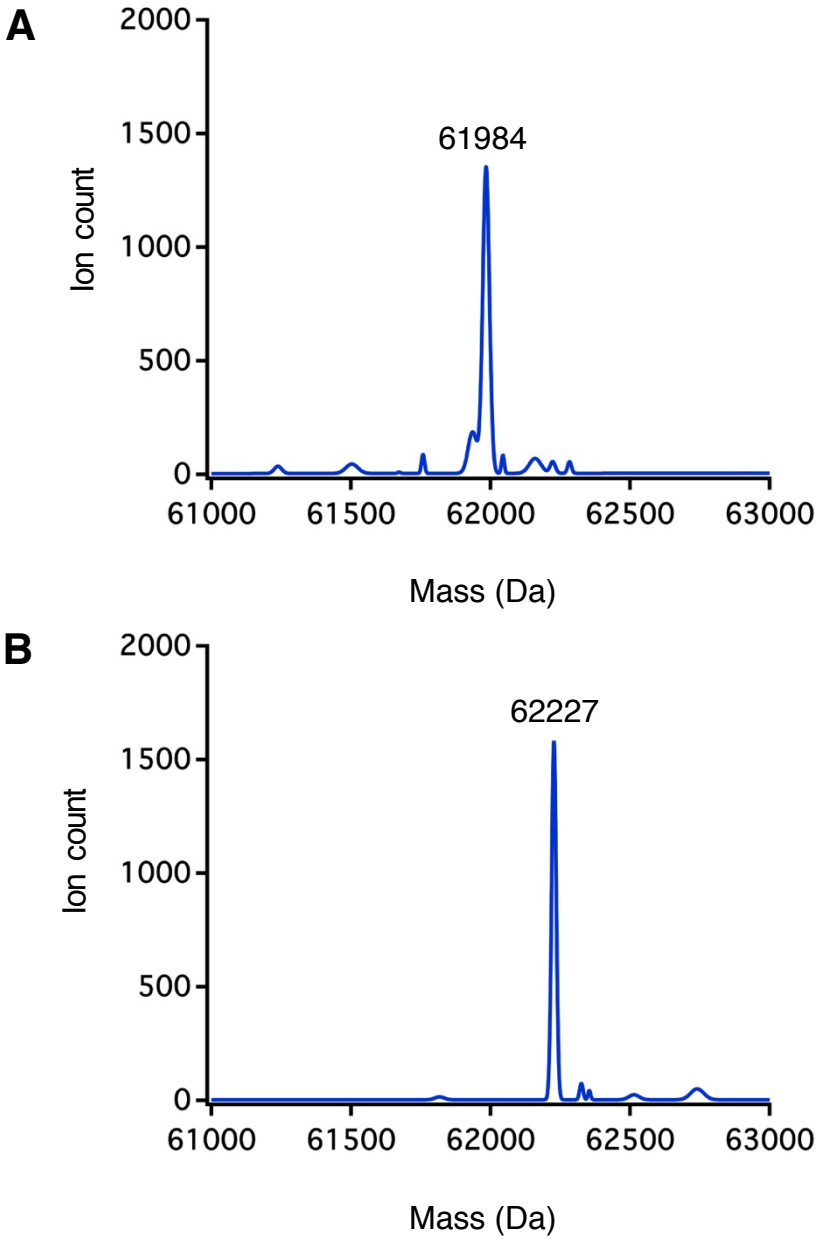


Figure S3. Deconvoluted mass spectra of Tar. (A) Tar isolated from cells expressing both NMT and Tar but not labeled with **1**. The mass at 61984 Da corresponds to unmodified Tar. (B) Tar isolated from cells labeled with **1**. The mass at 62227 Da corresponds to Tar modified with **1** (sodium adduct). Calculated and observed masses for modification of Tar are listed in Table S4.

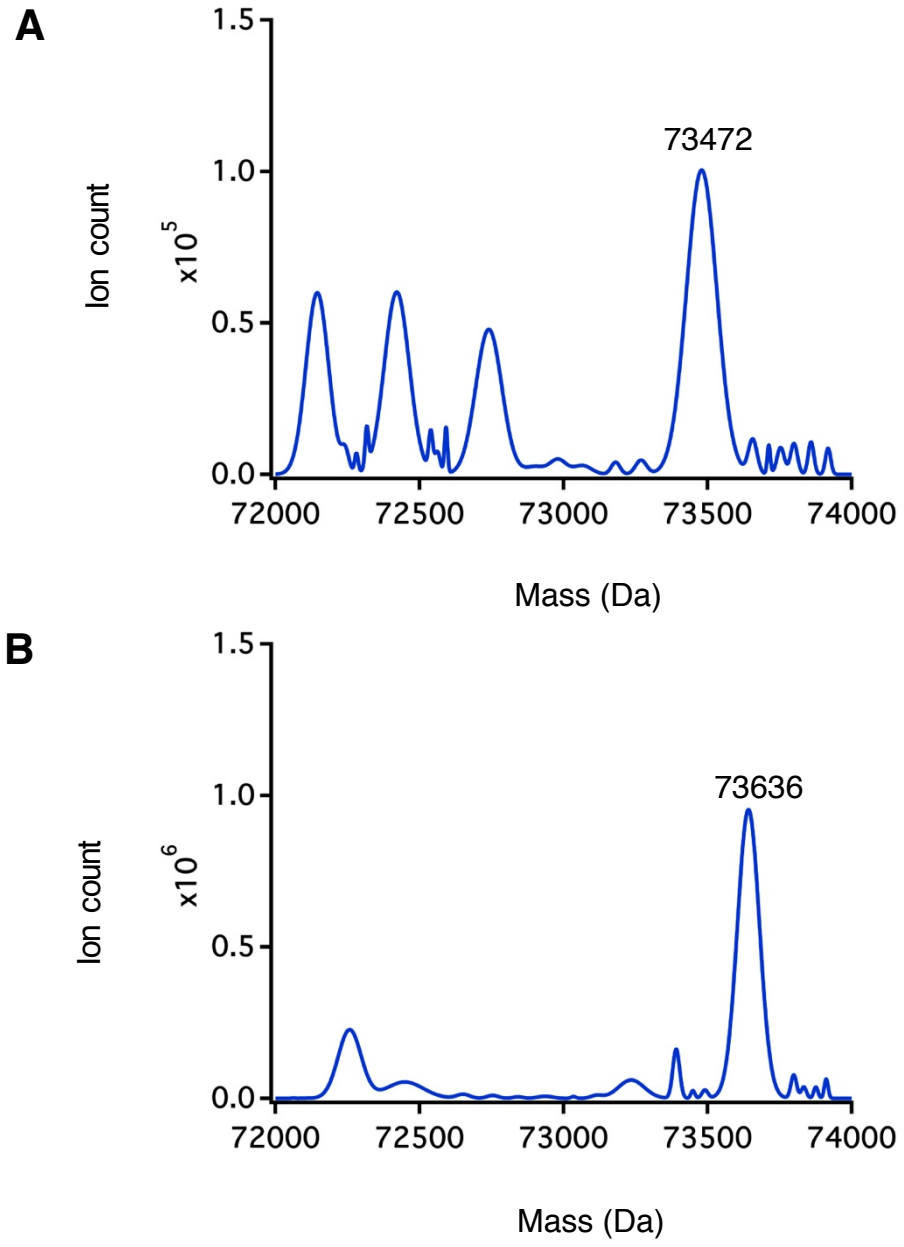


Figure S4. Deconvoluted mass spectra of CheA. (A) CheA isolated from cells expressing both NMT and CheA but not labeled with **1**. The mass at 73472 Da corresponds to the phosphorylated form of CheA. (B) CheA isolated from cells labeled with **1**. The mass at 73636 Da corresponds to CheA modified with **1** (sodium adduct). Calculated and observed masses for modification of CheA are listed in Table S5.

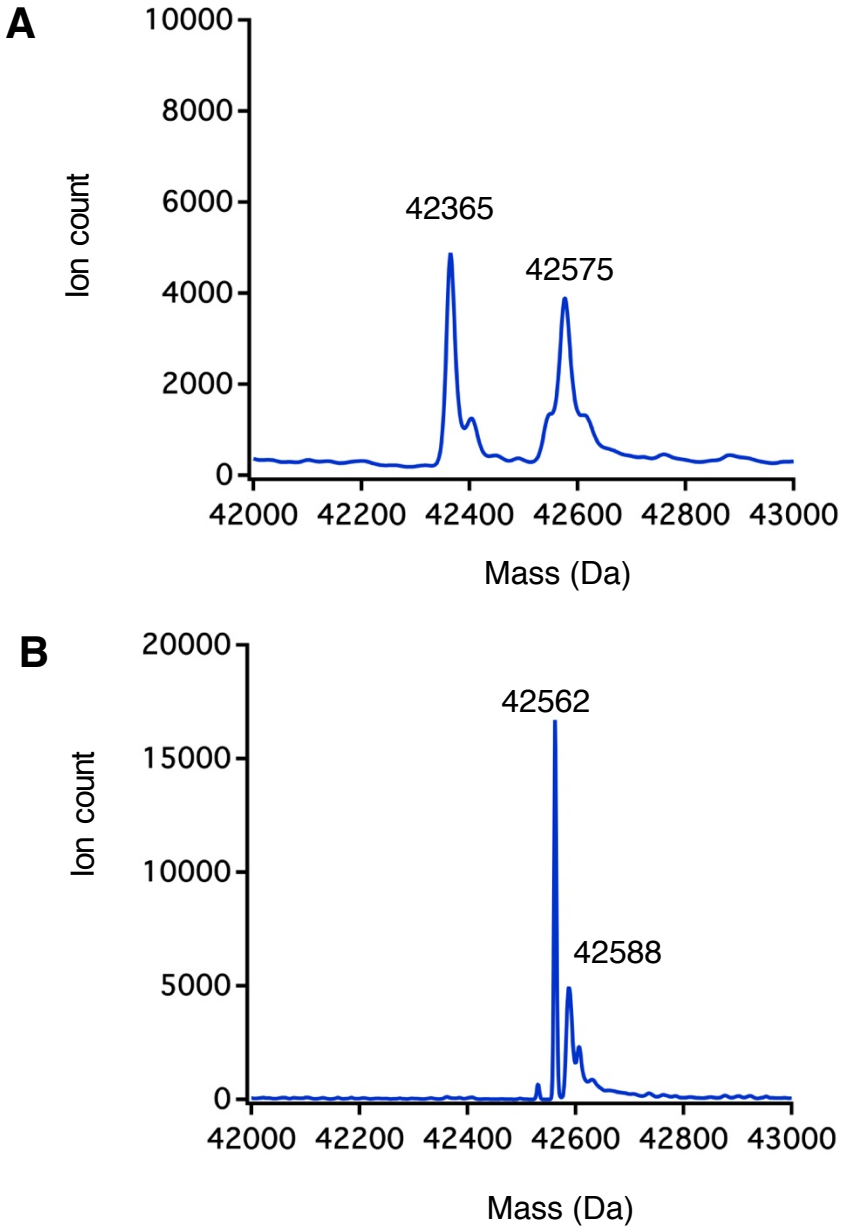


Figure S5. Deconvoluted mass spectra of FtsZ. (A) FtsZ isolated from cells expressing both NMT and FtsZ but not labeled with **1**. The mass at 42365 Da corresponds to unmodified FtsZ, and the mass at 42575 Da corresponds to FtsZ modified from endogenous myristic acid. (B) FtsZ isolated from cells labeled with **1**. The mass at 42588 Da corresponds to FtsZ modified with **1**. The mass at 42562 Da corresponds to FtsZ modified with **1**, with reduction of the terminal azide to an amine. Calculated and observed masses for modification of FtsZ are listed in Table S6.

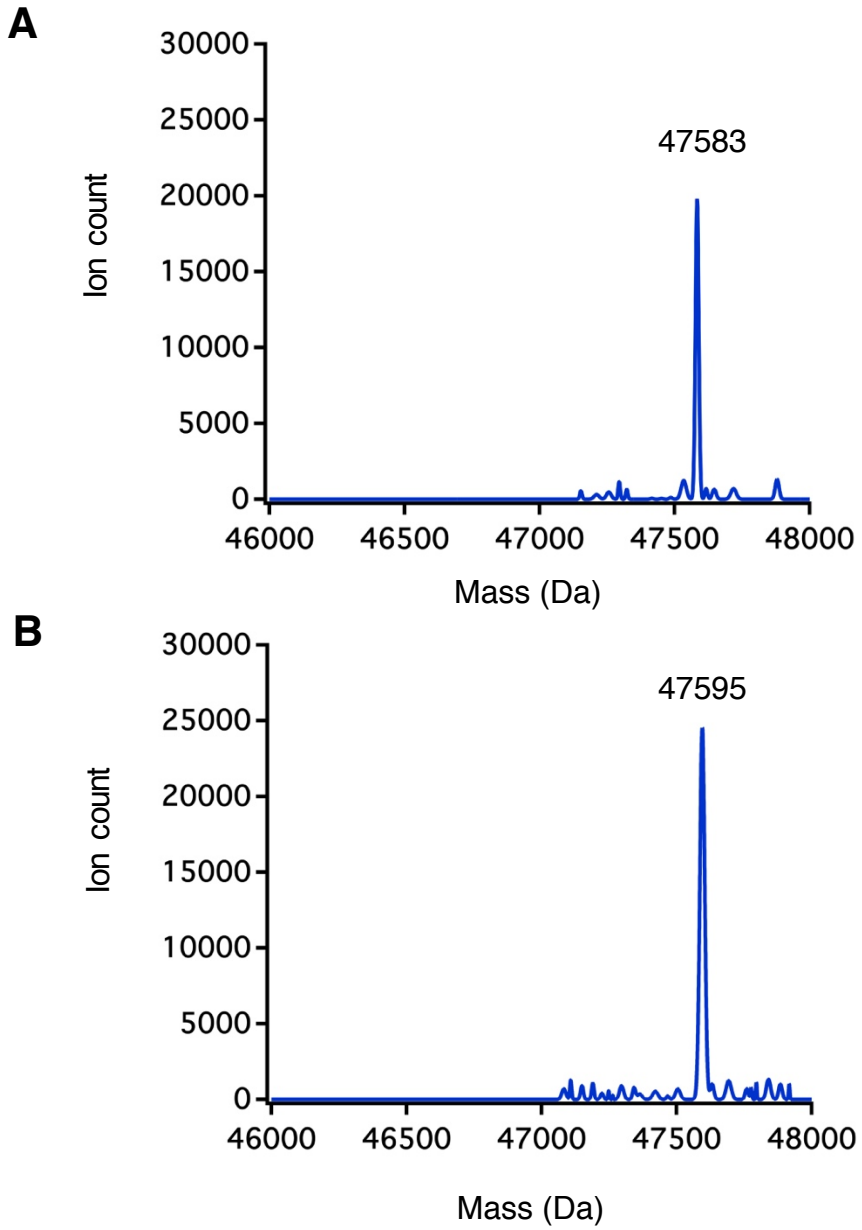


Figure S6. Deconvoluted mass spectra of FtsA. (A) FtsA isolated from cells expressing both NMT and FtsA but not labeled with **1**. The mass at 47583 Da corresponds to FtsA modified from endogenous myristic acid. (B) FtsA isolated from cells labeled with **1**. The mass at 47595 Da corresponds to FtsA modified with **1**. Calculated and observed masses for modification of FtsA are listed in Table S7.

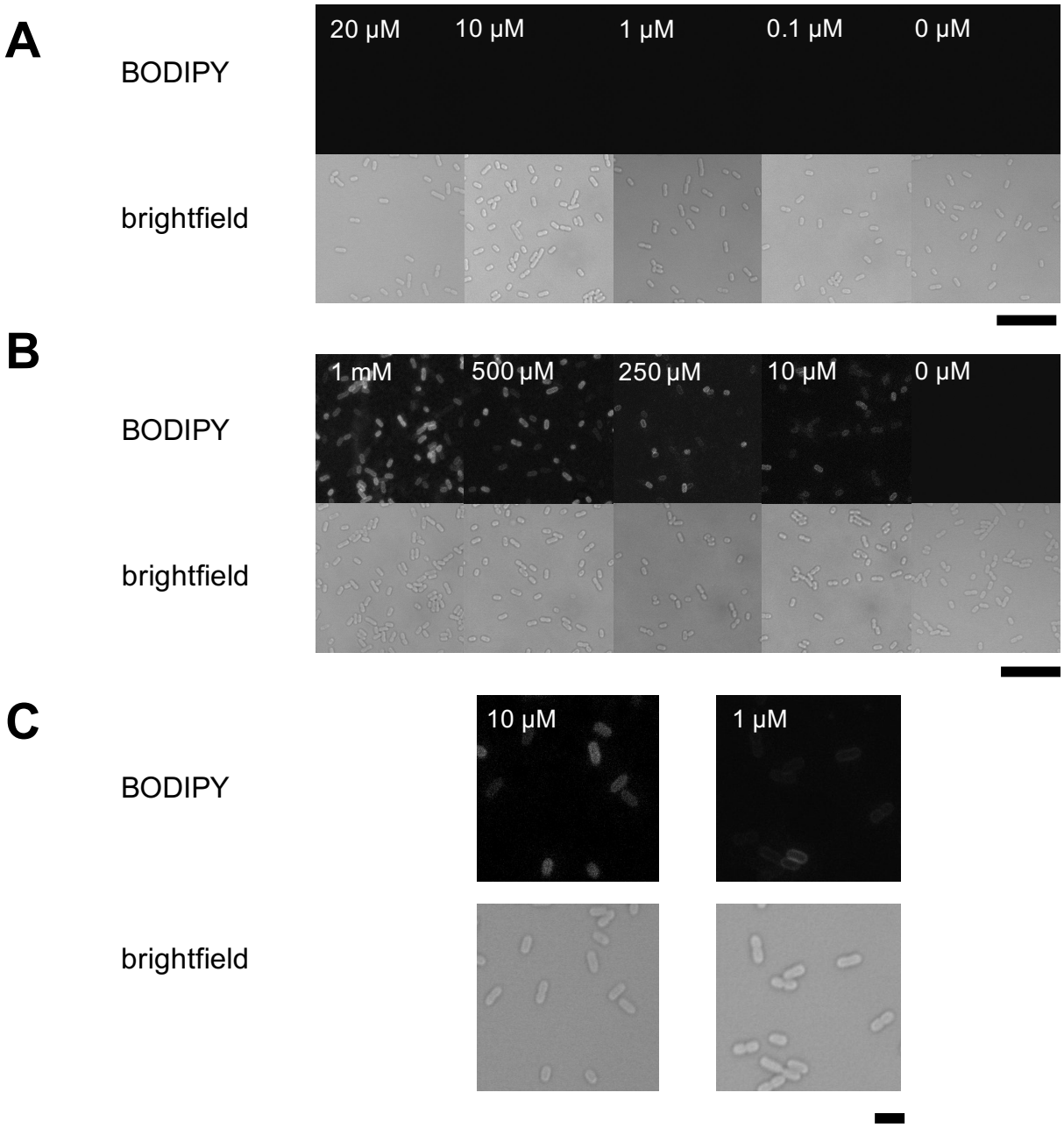


Figure S7. **Screening fatty acid and fluorophore concentration ranges.** Cells not expressing a target protein were grown at 37 °C to OD_{600} = 0.5 and treated as described in (A) – (C). After treatment, cells were washed three times with PBS and imaged by confocal fluorescence microscopy. (A) Cells that were not treated with **1** were labeled with different concentrations of **2** for 30 min at 37 °C. Scale bar = 10 µm. (B) Cells that were treated for 2 h with different concentrations of **1** were labeled with 20 µM **2**. Scale bar = 10 µm. (C) Cells that were treated for 2 h with 250 µM **1** and labeled for 30 min with different concentrations of **2**. Fluorescence can be observed for 10 µM **2** and low fluorescence is observed for 1 µM **2**. Scale bar = 2 µm.

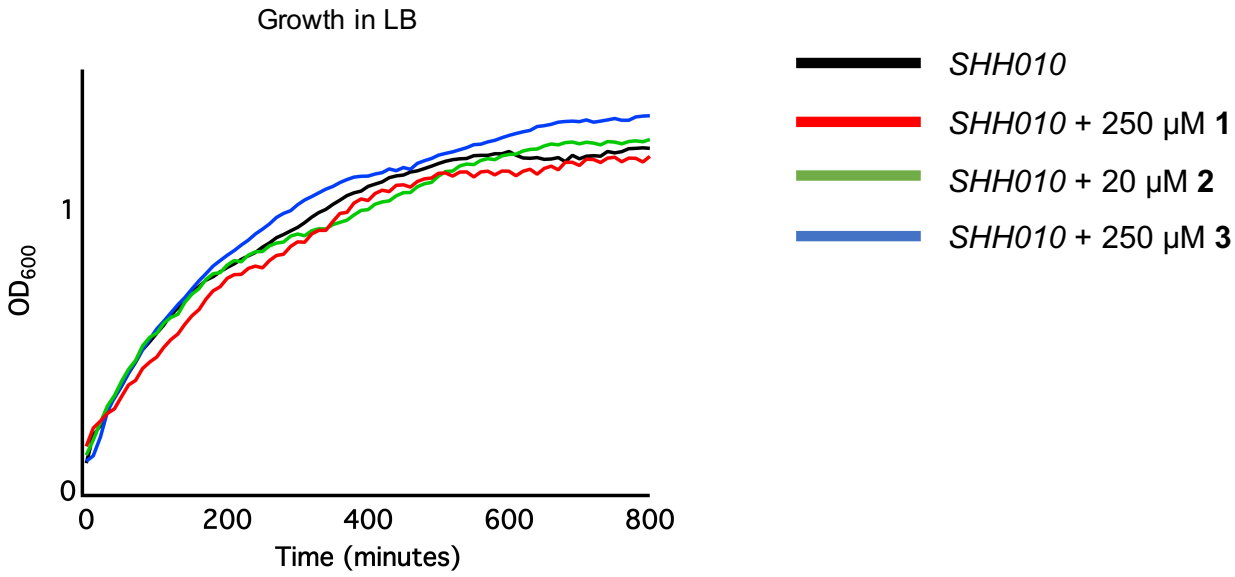


Figure S8. Addition of 1, 2, or 3 does not affect cell growth. Cells were diluted from an overnight culture to an OD_{600} = 0.1. Cells were then treated with either **1, 2,** or **3,** and continued to grow through the course of 800 min. OD_{600} was monitored every 10 min for each culture. *E. coli* strain SHH010 (culture bearing plasmids pBAD24-hCaNB-CheA-myc and pHV738-NMT-MetAP) was used in this experiment.

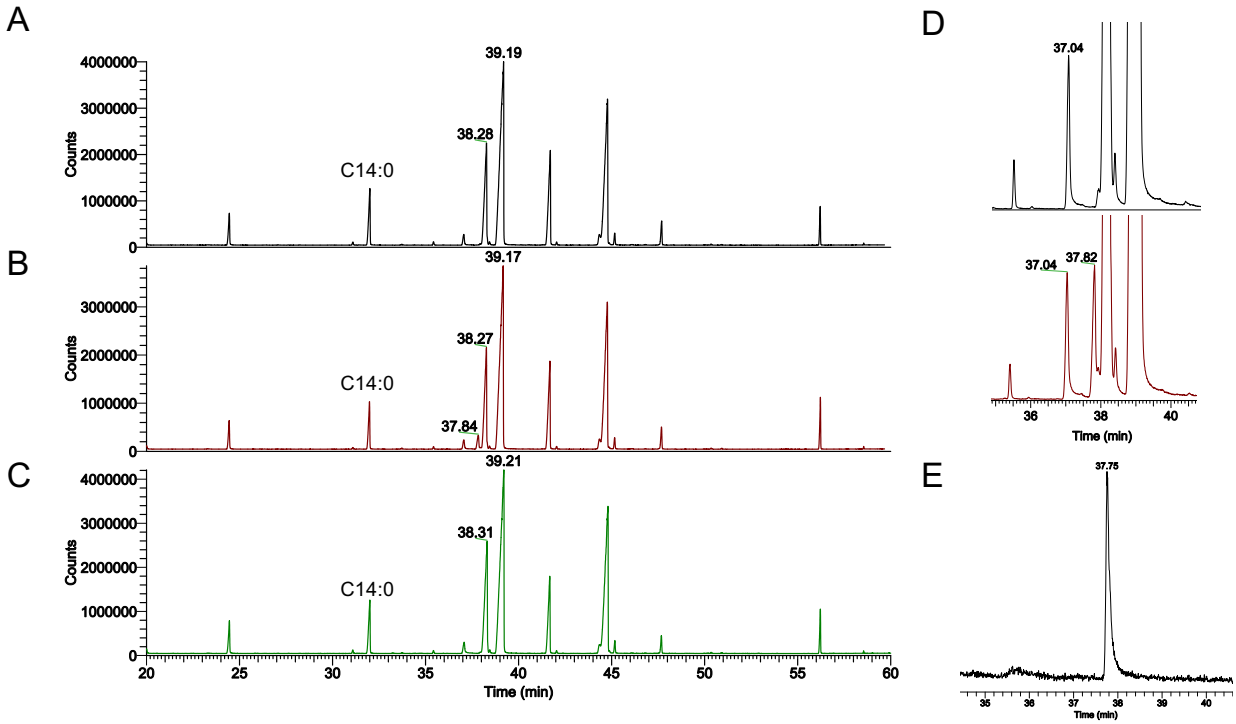


Figure S9. GC traces of fatty acid methyl esters (FAMEs) extracted from *E. coli*. FAMEs were extracted from cells that were (A) untreated with fatty acids, (B) treated with **1**, or (C) treated with **3**. The methyl ester derivative of **1** is found in (B) at t = 37.84 min. (D) Zoomed image comparing FAMEs from untreated cells (top) and cells treated with **1** (bottom). For cells treated with **3**, the fatty acid pool is essentially identical to that of cells that were not treated with any azido-fatty acids. (E) Compound **1** was subjected to esterification and injected for GC–MS analysis. The elution time (t = 37.75 min) matches that of the peak in trace (B) at t = 37.84 min. The methyl ester of endogenous myristic acid (C14:0) is labeled in traces A–C. FAME analysis yields no evidence that probes **1** and **3** undergo chain-length redistribution.

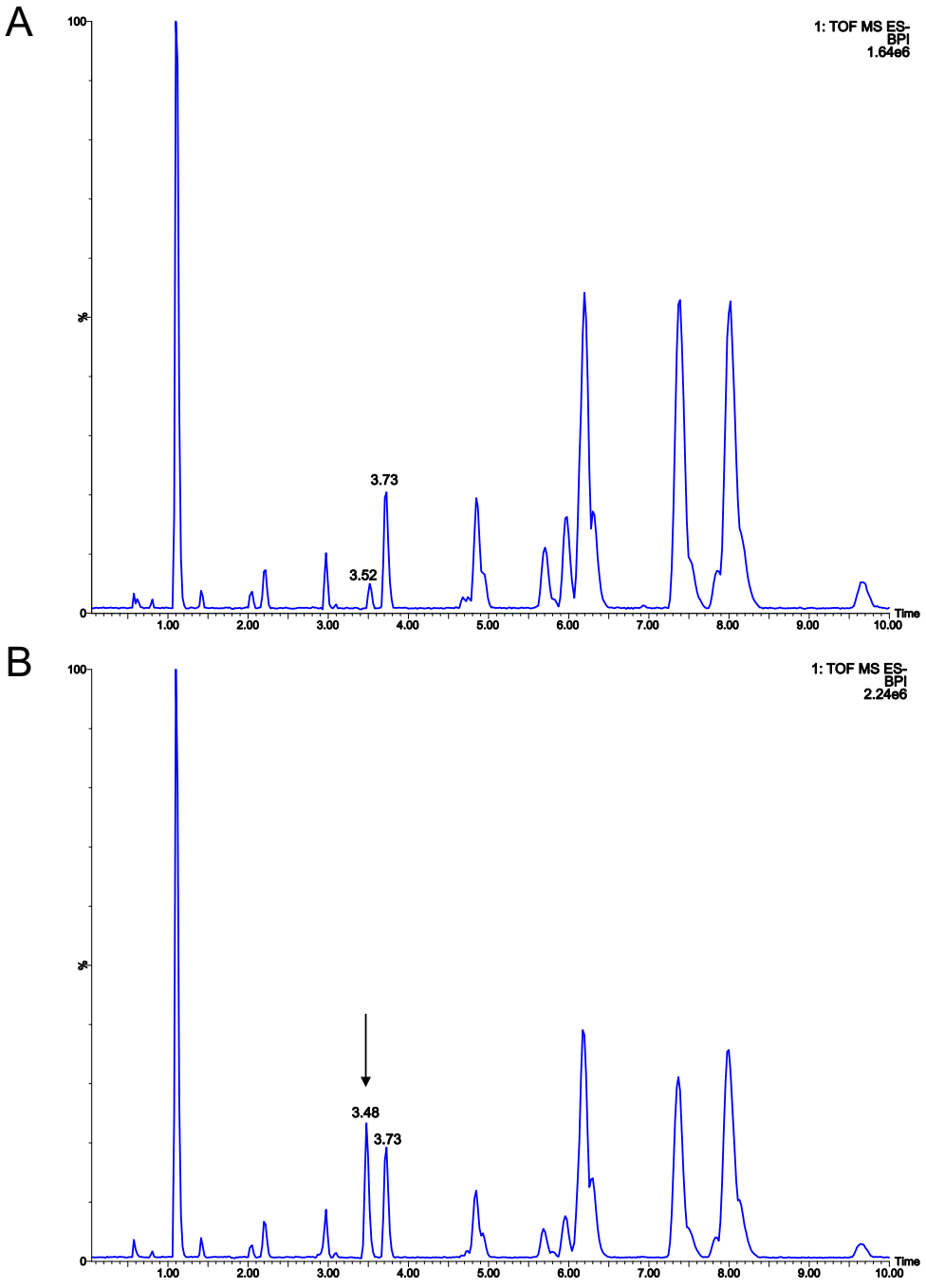


Figure S10. LC traces of intact lipids from *E. coli*. Intact lipids were extracted from cells (A) untreated with azide-containing fatty acids or (B) treated with **1**. (B) Fatty acid probe **1** is incorporated into a phosphatidylethanolamine with a C_{16} acyl chain (t = 3.48 min). Identification of PE molecule eluting at t = 3.48 min was performed by analysis of fragmentation patterns in both negative (Figure S11) and positive (Figure S12) mode. BPI = Base peak intensity.

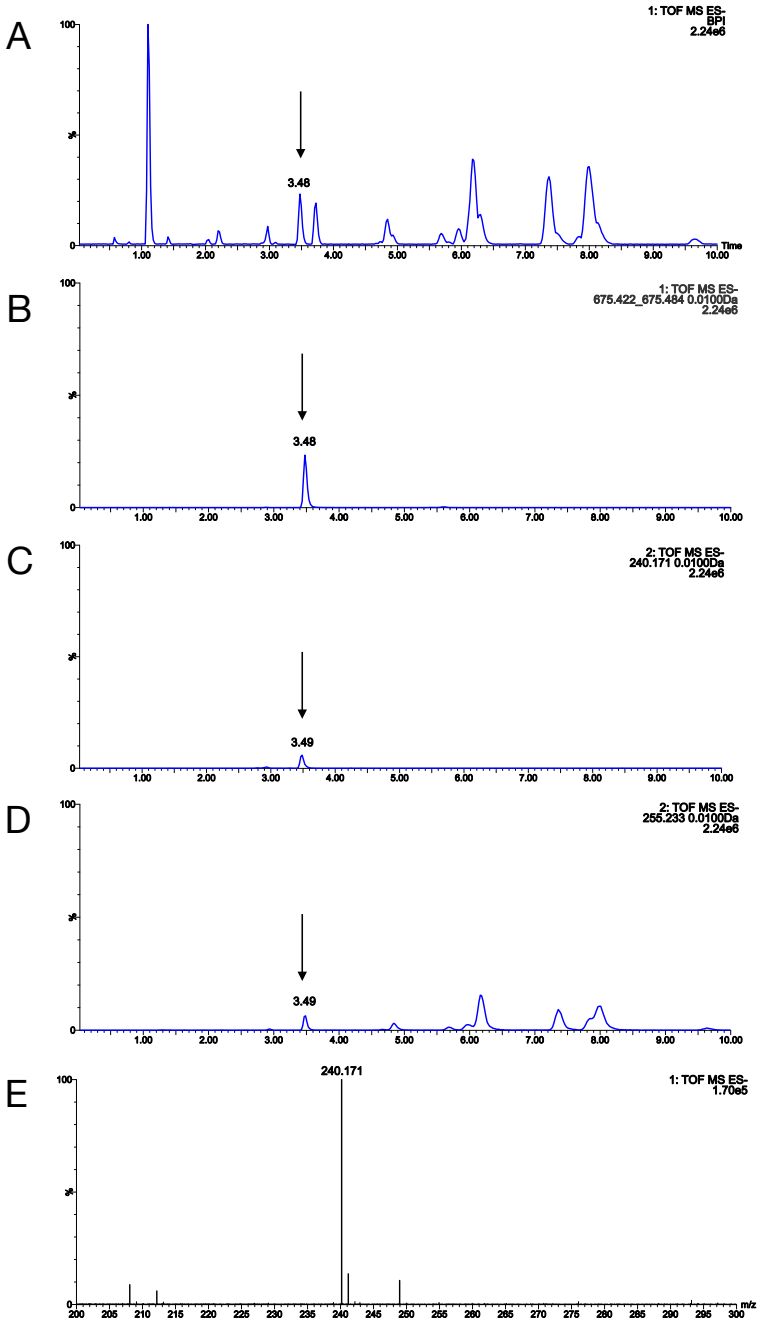


Figure S11. Identification of fatty acids from phospholipids (negative mode). (A) Base peak ion (BPI) chromatogram of cells treated with **1**. (B) Extracted ion chromatogram (EIC) for phospholipid (parent ion), using m/z value search from MS1 channel (m/z = 675.422 – 675.484 ± 0.010 Da). Elution peak occurs at t = 3.48 min. (C and D) Identification of individual chain components of phospholipid eluting at t = 3.48 min. EICs were generated using m/z values from MS2 channel (fragment ions). (C) EIC for m/z value corresponding to **1** (m/z = 240.171 ± 0.010 Da). (D) EIC for m/z value corresponding to palmitic acid (m/z = 255.233 ± 0.010 Da). The fragment ions (traces C and D) show an elution peak at t = 3.48 min, matching that of the peak corresponding to the parent ion (trace B) and indicating that **1** is incorporated into a cellular lipid bearing a palmitic acid chain. Max ion count for traces A–D: 2.24 x 10^6. (E) Probe **1** was run to confirm production of the expected ion at the expected m/z.

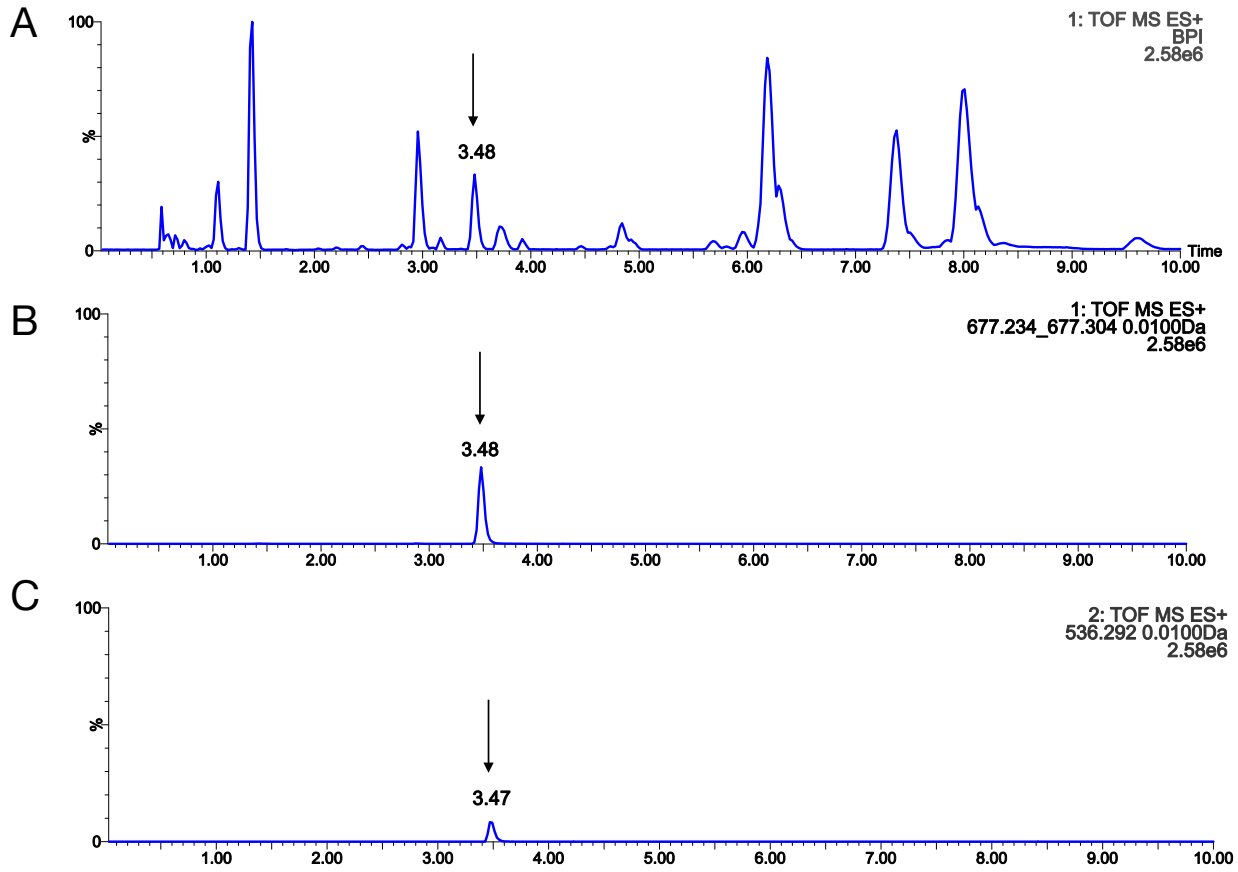


Figure S12. Identification of fatty acids from phospholipids (positive mode). (A) Base peak ion (BPI) chromatogram of cells treated with **1**. (B) Extracted ion chromatogram (EIC) for phospholipid (parent ion), using m/z value search from MS1 channel (m/z = 677.234 – 677.304 ± 0.010 Da). (C) EIC for the fragment ion (MS2 channel; m/z = 536.292 ± 0.010 Da) shows an elution peak at t = 3.48 min. The difference in m/z between the parent ion in (B) and fragment ion in (C) suggests the loss of phosphorylethanolamine (calculated m/z: 141.019 Da), consistent with formation of a phosphatidylethanolamine bearing acyl chains derived from **1** and palmitic acid. Max ion count for traces A–C: 2.58 x 10^6.

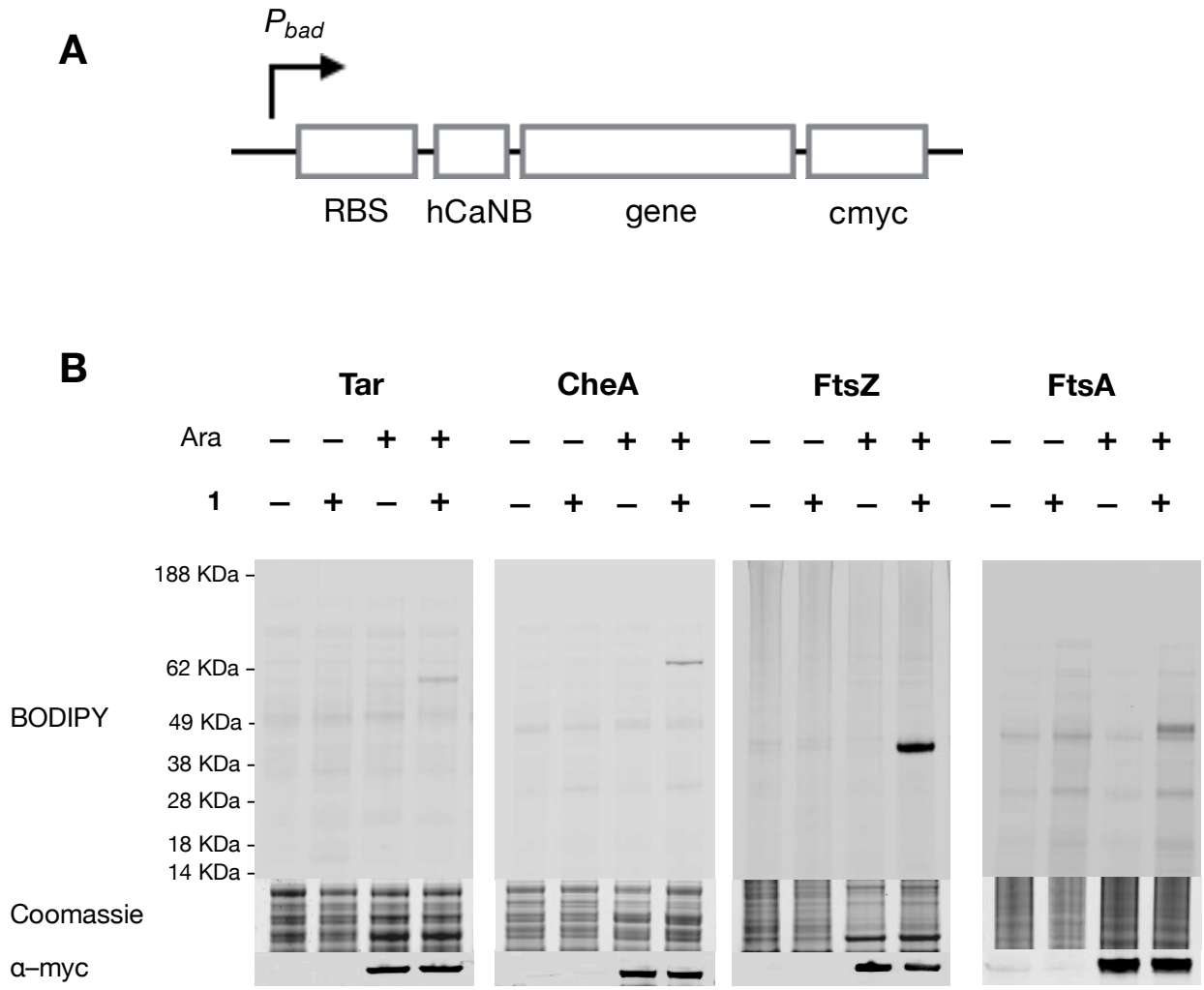


Figure S13. **In-gel fluorescence detection of proteins expressed from pBAD24 plasmids and labeled with 1.** (A) The gene encoding the protein of interest is placed under control of the P_{BAD} promoter in the modified pBAD24 plasmid. The protein is outfitted with the *N*-terminal nonapeptide sequence from calcineurin B (hCaNB) for NMT recognition and a *C*-terminal myc-tag (cmyc) for immunoblotting and immunofluorescence imaging. RBS = ribosome binding site. (B) In-gel fluorescence analysis shows NMT can achieve site-specific labeling of *E. coli* proteins expressed under control of the arabinose-inducible promoter. SDS–PAGE analysis of *E. coli* lysates. Protein expression was achieved by addition of 0.2% w/v L-arabinose (Ara). Cultures were labeled with **1** when protein expression was induced. Cells were lysed and lysates were treated with **2**. Western blot analysis against a *C*-terminal myc tag with a primary antibody conjugated to Alexa Fluor 647 confirms protein expression.

The T5 promoter can exhibit basal expression in the absence of an inducer. The pBAD promoter is more tightly regulated with reduced basal expression. In cells outfitted with pBAD24 plasmids, NMT is constitutively expressed under control of the *tac* promoter. We found sufficient labeling for imaging with basal NMT expression. For a comparison of expression systems, see Ref. 9.

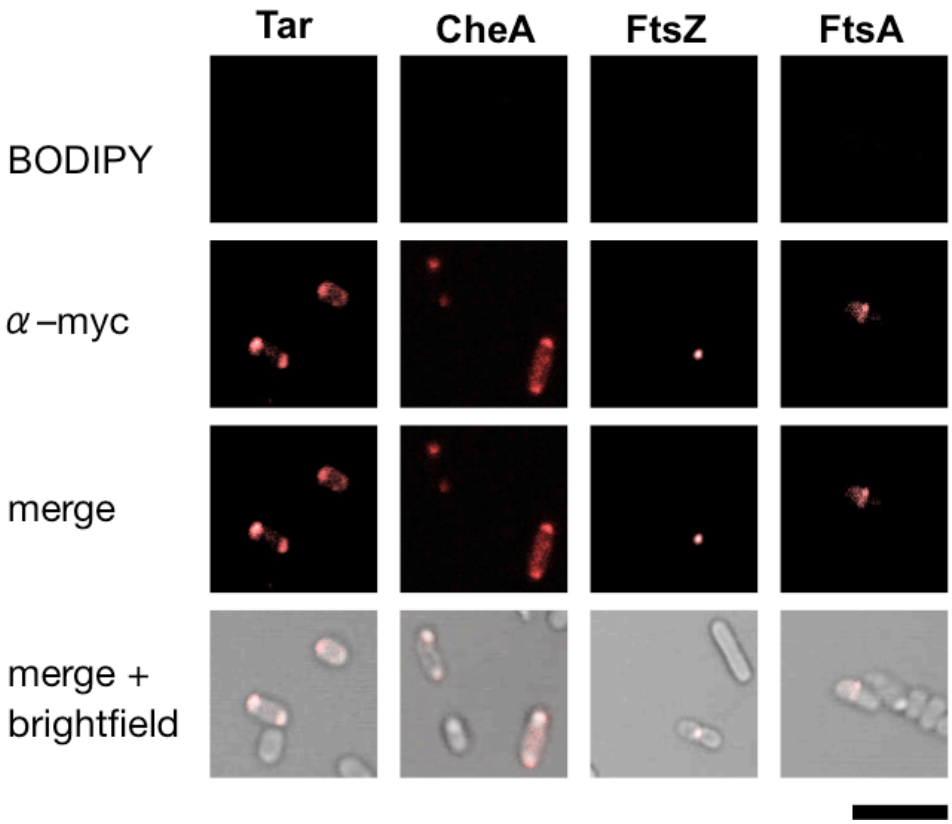


Figure S14. **Immunofluorescence labeling of bacterial proteins.** Bacterial proteins that are not labeled with **1** show localization patterns indistinguishable from those treated with **1**. Polar localization is observed for Tar and CheA; septal localization for FtsZ and FtsA. After protein expression was induced, cells were fixed, permeabilized, and treated with **2** and an anti–myc antibody conjugated to Alexa Fluor 647. (scale bar = 2 μm).

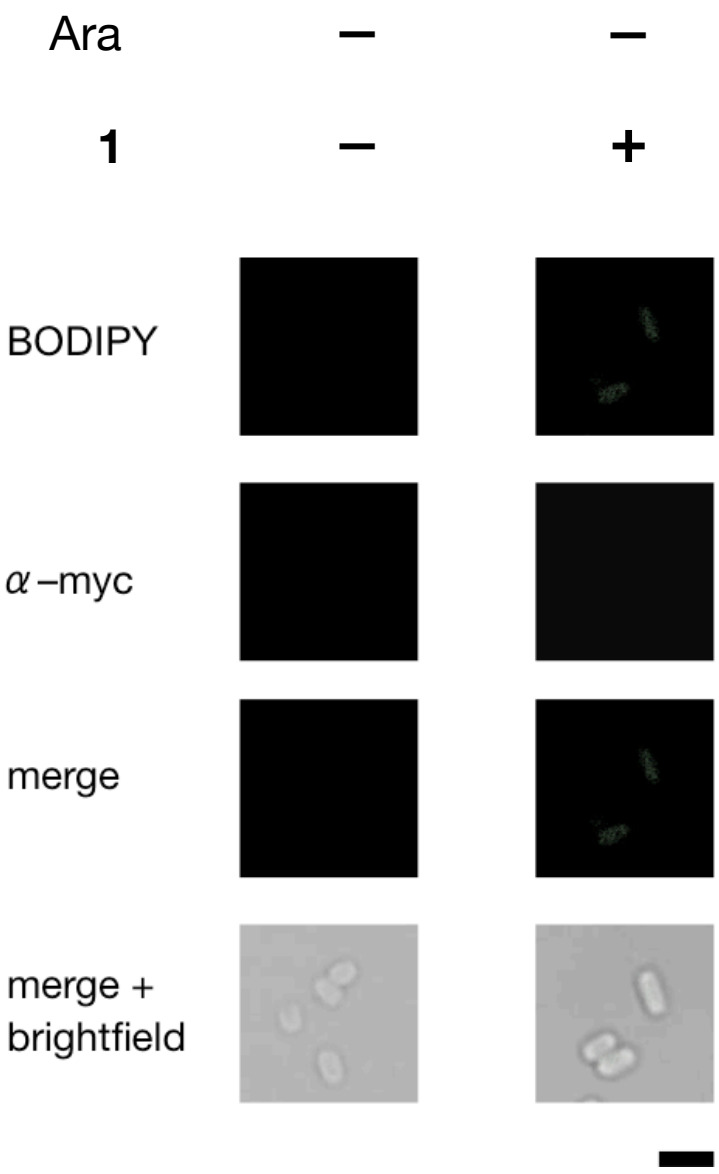


Figure S15. **Immunofluorescence labeling of uninduced cells or uninduced cells treated with 1.** Control experiments where cells do not express a target protein but are treated with **1, 2**, and antibody (following fixation and permeabilization) show no observable, distinct localization patterns (for all four proteins Tar, CheA, FtsZ, and FtsA), confirming that there is little interference from labeling of free **1** or of **1** incorporated into membrane lipids (scale bar = 1 μm).

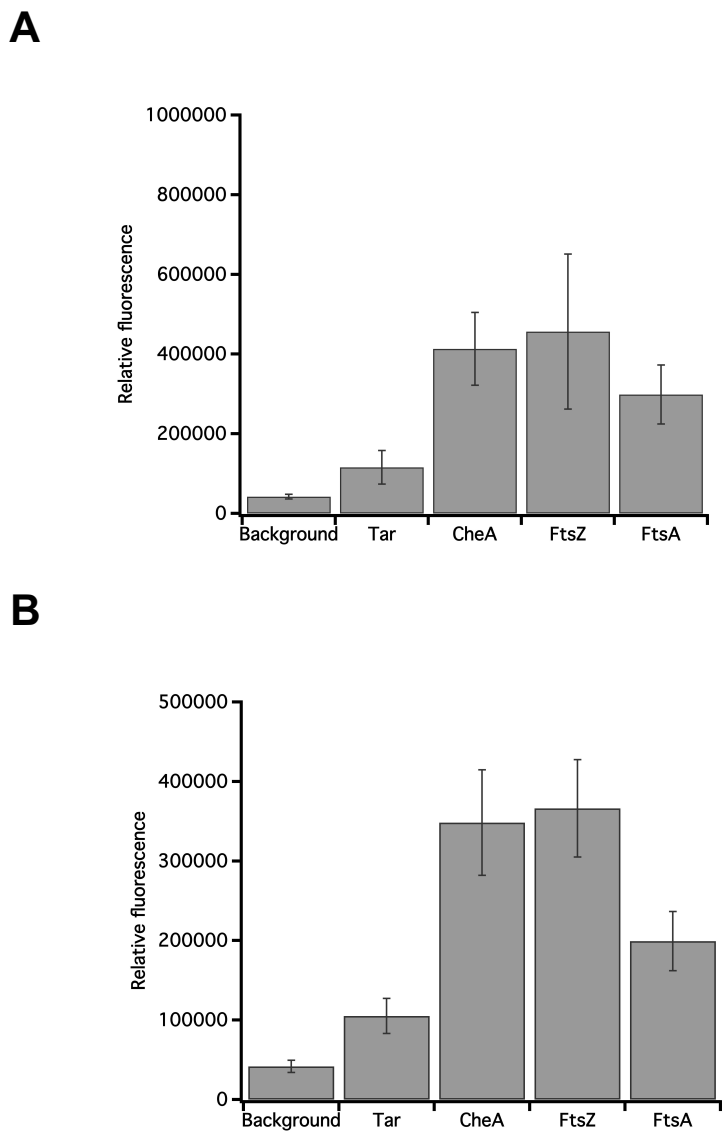


Figure S16. **Relative fluorescence intensities for live cells labeled with 1 or 3 and 2.** Fluorescence signal from cells was quantified using Cell Profiler. Background signal was measured from cells that were not expressing protein targets but were treated with 250 μM of either (A) **1** or (B) **3**, and labeled with 20 μM **2**. The fold changes above background for Tar, CheA, FtsZ, and FtsA are (A) 2.7, 9.8, 10.8, and 7.1 and (B) 2.5, 8.4, 8.8, and 4.8, respectively. Error bars denote standard deviation from fluorescence quantification for 100–150 cells for each of the cells expressing one of the four protein targets or cells used for quantifying background signal.

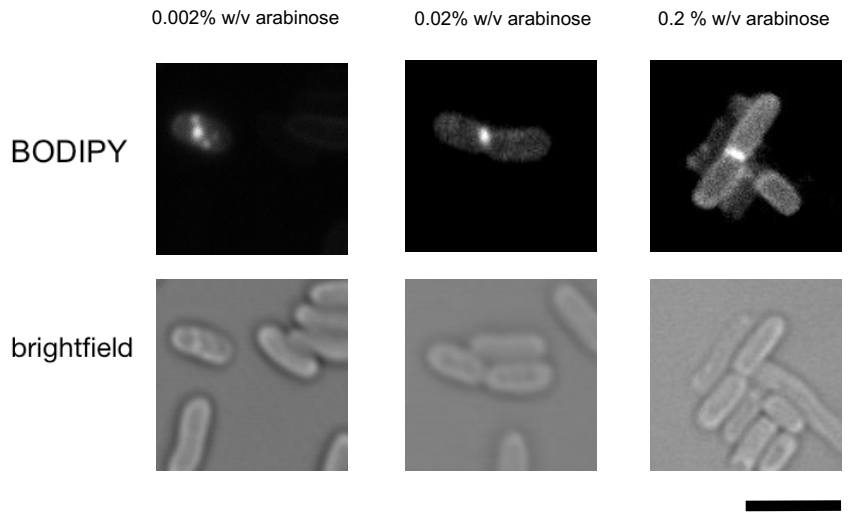


Figure S17. Cells can be labeled with lower concentrations of arabinose. We were able to achieve similar labeling when protein targets were induced with lower concentrations of arabinose. Shown here is a representative example of FtsZ expressing cells labeled with 250 μM **1** and 20 μM **2.** Scale bar is 2 μm.

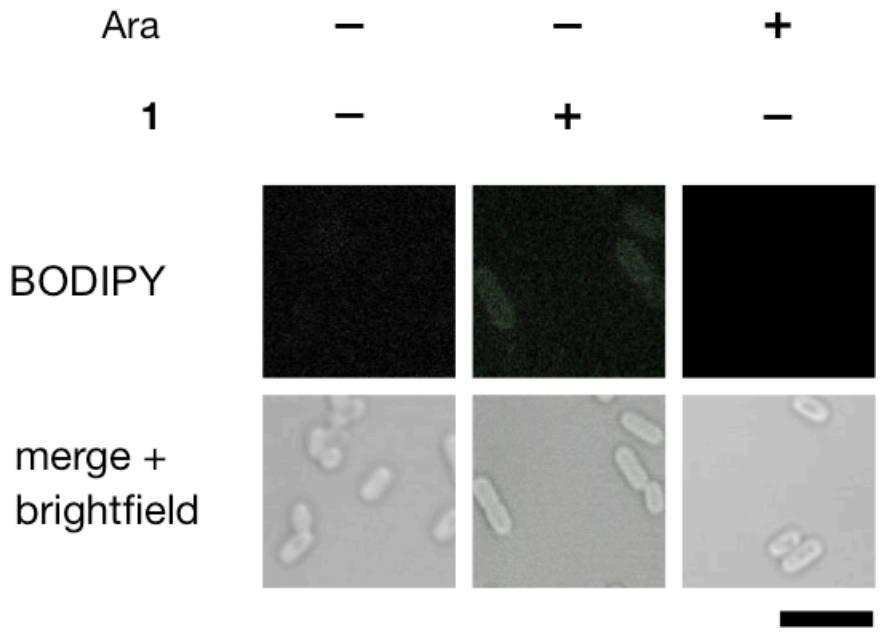


Figure S18. Live-cell fluorescence imaging of uninduced cells. Representative live-cell imaging for control experiments. Cells that do not express the protein of interest, but that have been treated with **1** and **2**, do not show distinct localization patterns. (scale bar = 1 μm).

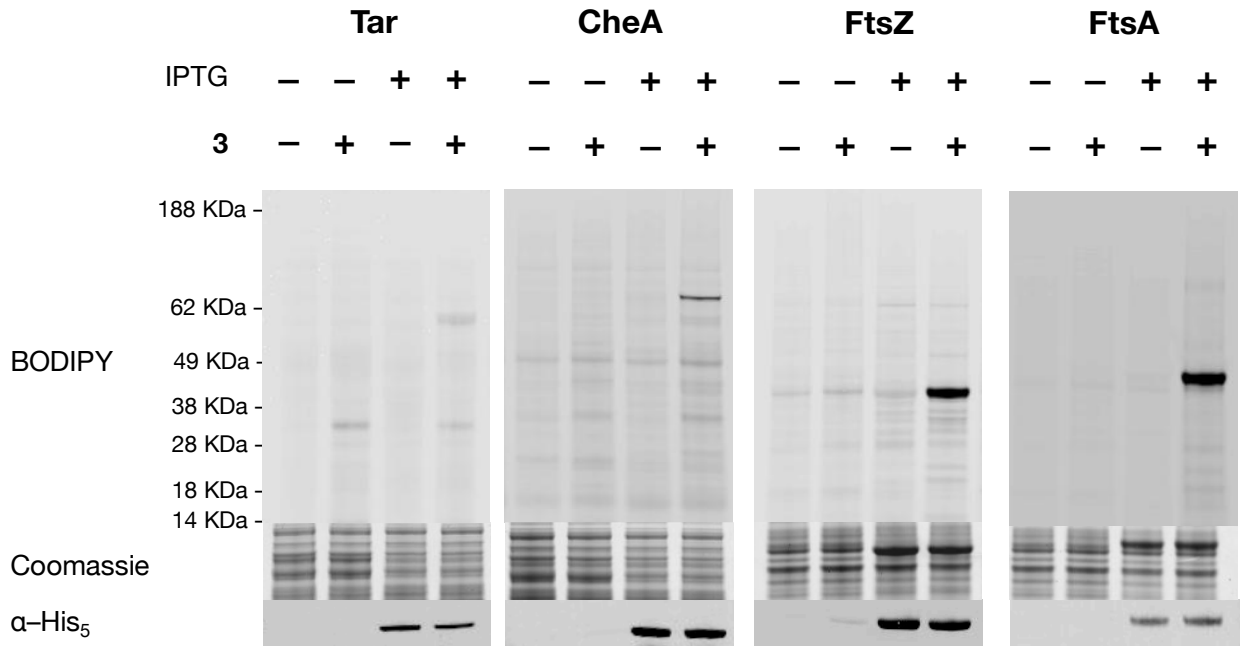


Figure S19. **In-gel fluorescence detection of proteins expressed from pQE80-L plasmids and labeled with 3.** SDS–PAGE analysis of *E. coli* lysates. Protein expression was achieved by addition of 1 mM IPTG. Cultures were labeled with **3** when protein expression was induced. Cells were lysed and lysates were treated with **2**. Western blot analysis against a *C*-terminal His_5 tag with a primary antibody conjugated to Alexa Fluor 647 confirms protein expression.

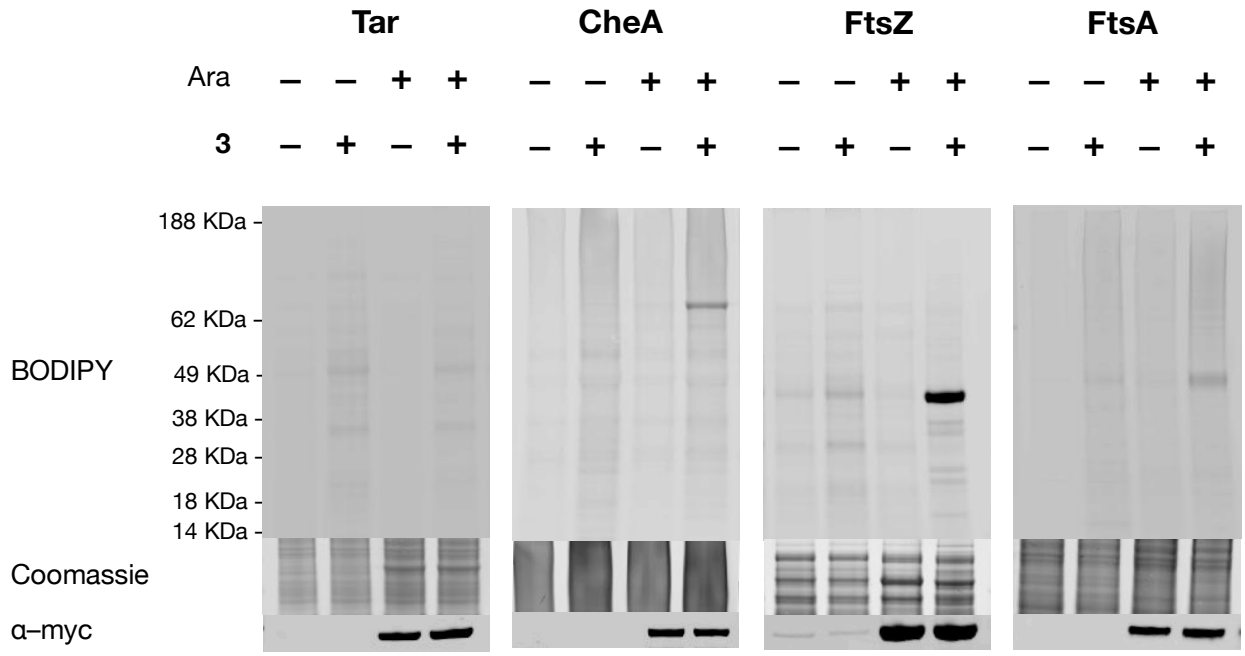


Figure S20. **In-gel fluorescence detection of proteins expressed from pBAD24 plasmids and labeled with 3.** SDS–PAGE analysis of *E. coli* lysates. Protein expression was achieved by addition of 0.2% w/v L-arabinose (Ara). Cultures were labeled with **3** when protein expression was induced. Cells were lysed and lysates were treated with **2**. Western blot analysis against a *C*-terminal myc tag with a primary antibody conjugated to Alexa Fluor 647 confirms protein expression. With hydrophilic fatty acid **3**, we were unable to observe fluorescence labeling for the transmembrane protein, Tar.

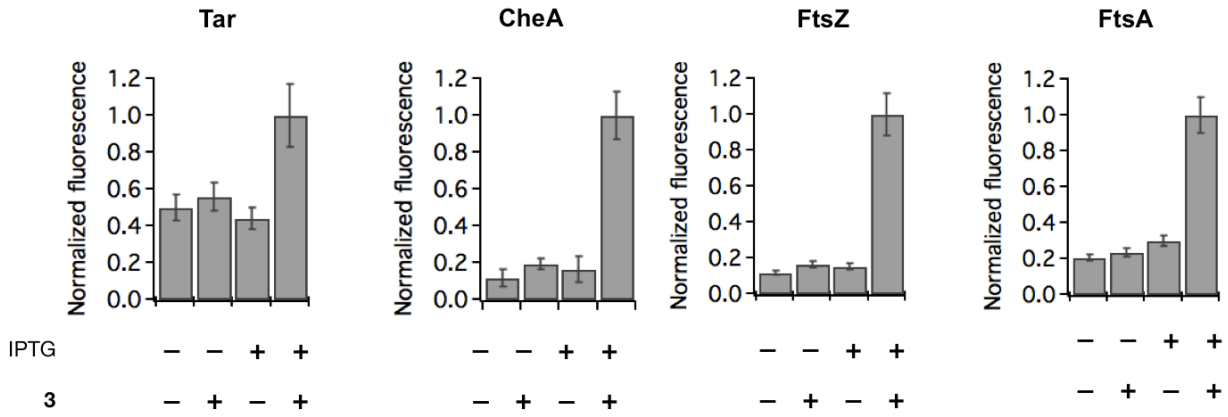


Figure S21. Fluorescence emission of proteins expressed from pQE80-L plasmids and labeled with 3. Crude lysates from cells (labeled with **3**) expressing one of the four bacterial proteins (Tar, CheA, FtsZ, or FtsA) were treated with **2** and separated using SDS–PAGE. Fluorescence measurements (from gels corresponding to Figure S19) were normalized to the bands corresponding to the labeled proteins in the coomassie gels. The extent of fluorescence enhancement above background measured by gel electrophoresis was 1.8-, 5.1-, 6.2-, and 4.3-fold, respectively. Error bars denote standard deviations from three independent experiments.

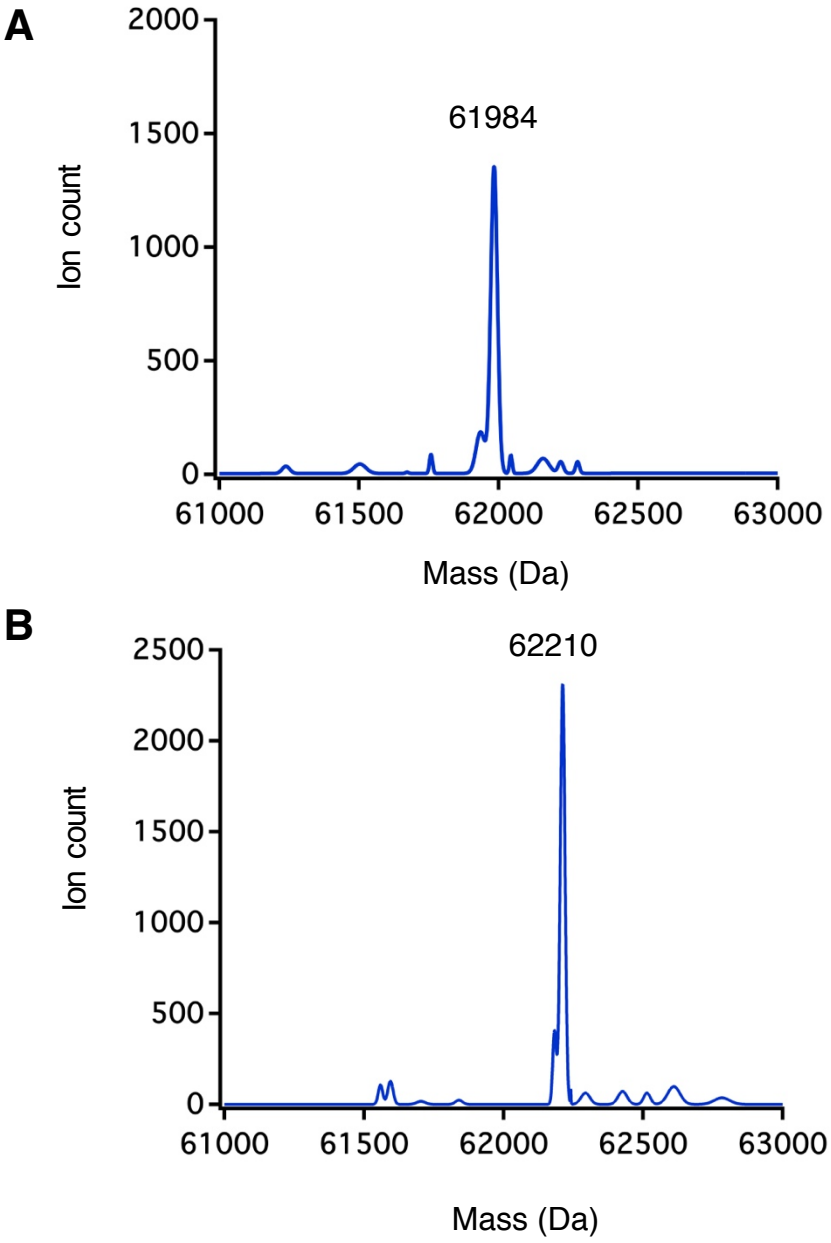


Figure S22. Deconvoluted mass spectra of Tar. (A) Tar isolated from cells expressing both NMT and Tar but not labeled with **3**. The mass at 61984 Da corresponds to unmodified Tar. (B) Tar isolated from cells labeled with **3**. The mass at 62210 Da corresponds to Tar modified with **3**. Calculated and observed masses for modification of Tar are listed in Table S4.

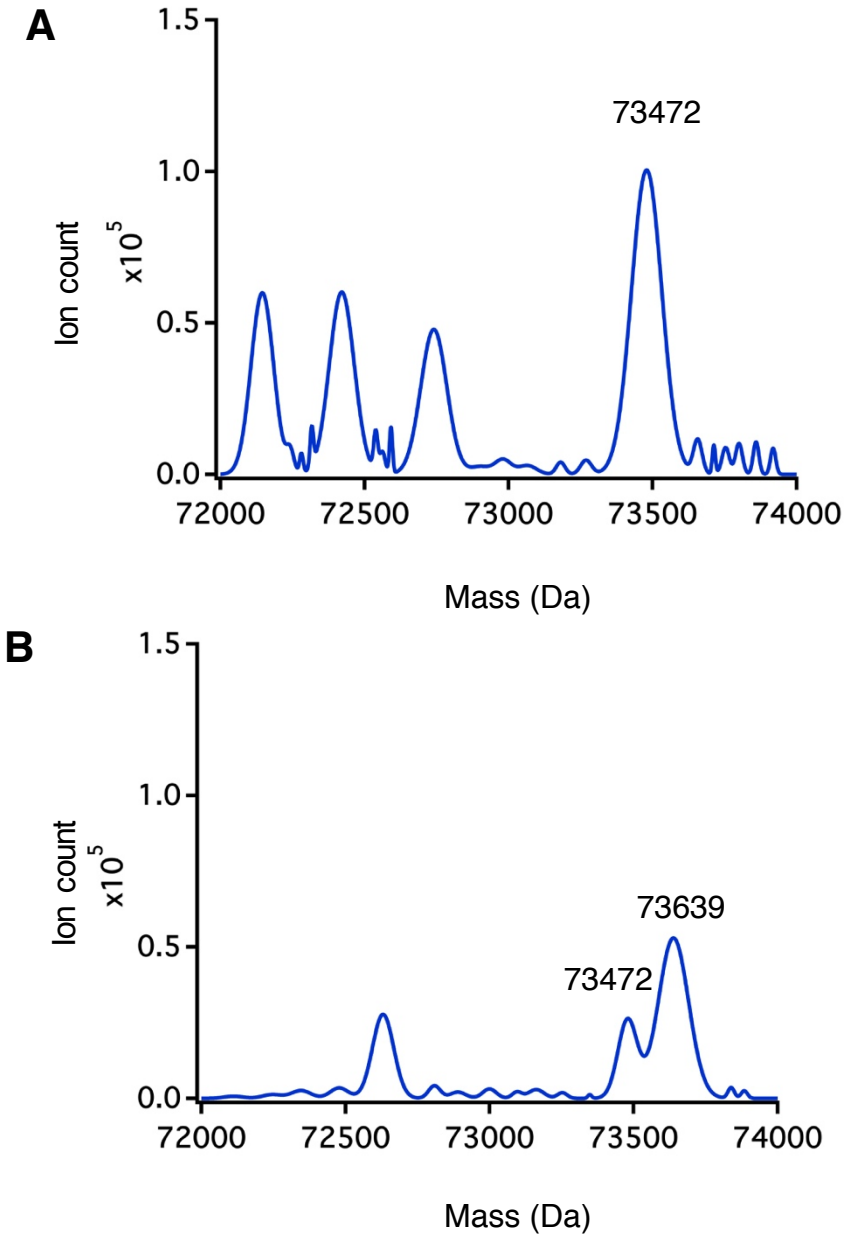


Figure S23. Deconvoluted mass spectra of CheA. (A) CheA isolated from cells expressing both NMT and CheA but not labeled with **3**. The mass at 73472 Da corresponds to the phosphorylated form of CheA. (B) CheA isolated from cells labeled with **3**. The mass at 73639 Da corresponds to CheA modified with **3** (sodium adduct). Calculated and observed masses for modification of CheA are listed in Table S5.

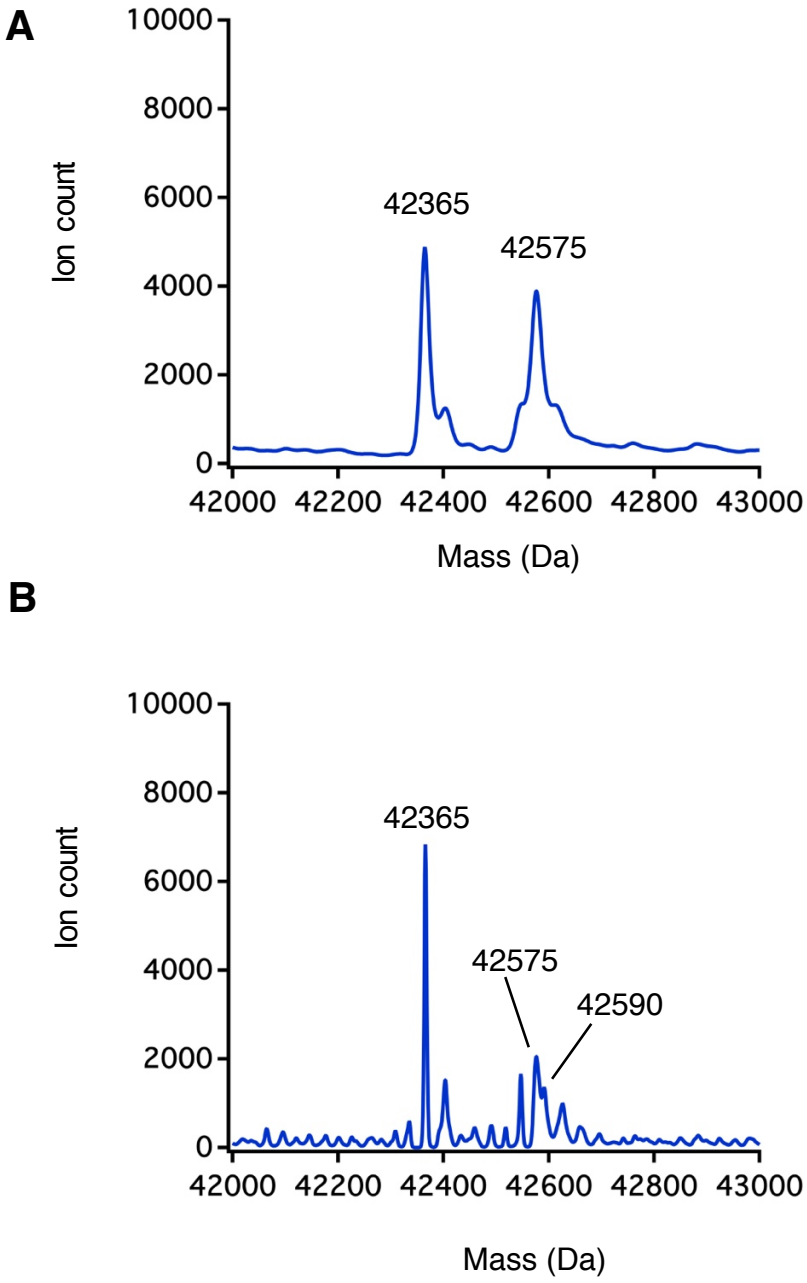


Figure S24. Deconvoluted mass spectra of FtsZ. (A) FtsZ isolated from cells expressing both NMT and FtsZ but not labeled with **3**. The mass at 42365 Da corresponds to unmodified FtsZ, and the mass at 42575 Da corresponds to FtsZ modified from endogenous myristic acid. (B) FtsZ isolated from cells labeled with **3**. The mass at 42590 Da corresponds to FtsZ modified with **3**. Calculated and observed masses for modification of FtsZ are listed in Table S6.

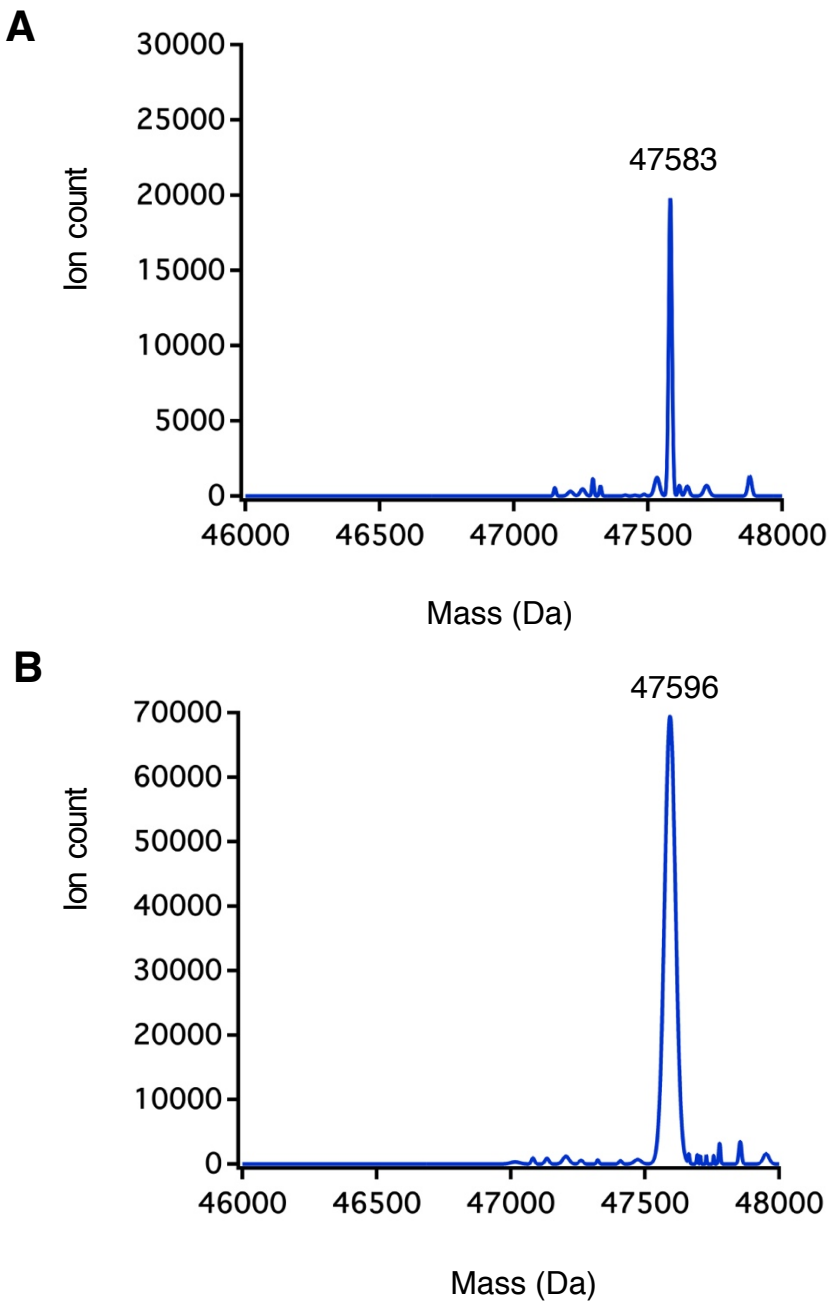


Figure S25. Deconvoluted mass spectra of FtsA. (A) FtsA isolated from cells expressing both NMT and FtsA but not labeled with **3**. The mass at 47583 Da corresponds to FtsA modified from endogenous myristic acid. (B) FtsA isolated from cells labeled with **3**. The mass at 47596 Da corresponds to FtsA modified with **3**. Calculated and observed masses for modification of FtsA are listed in Table S7.

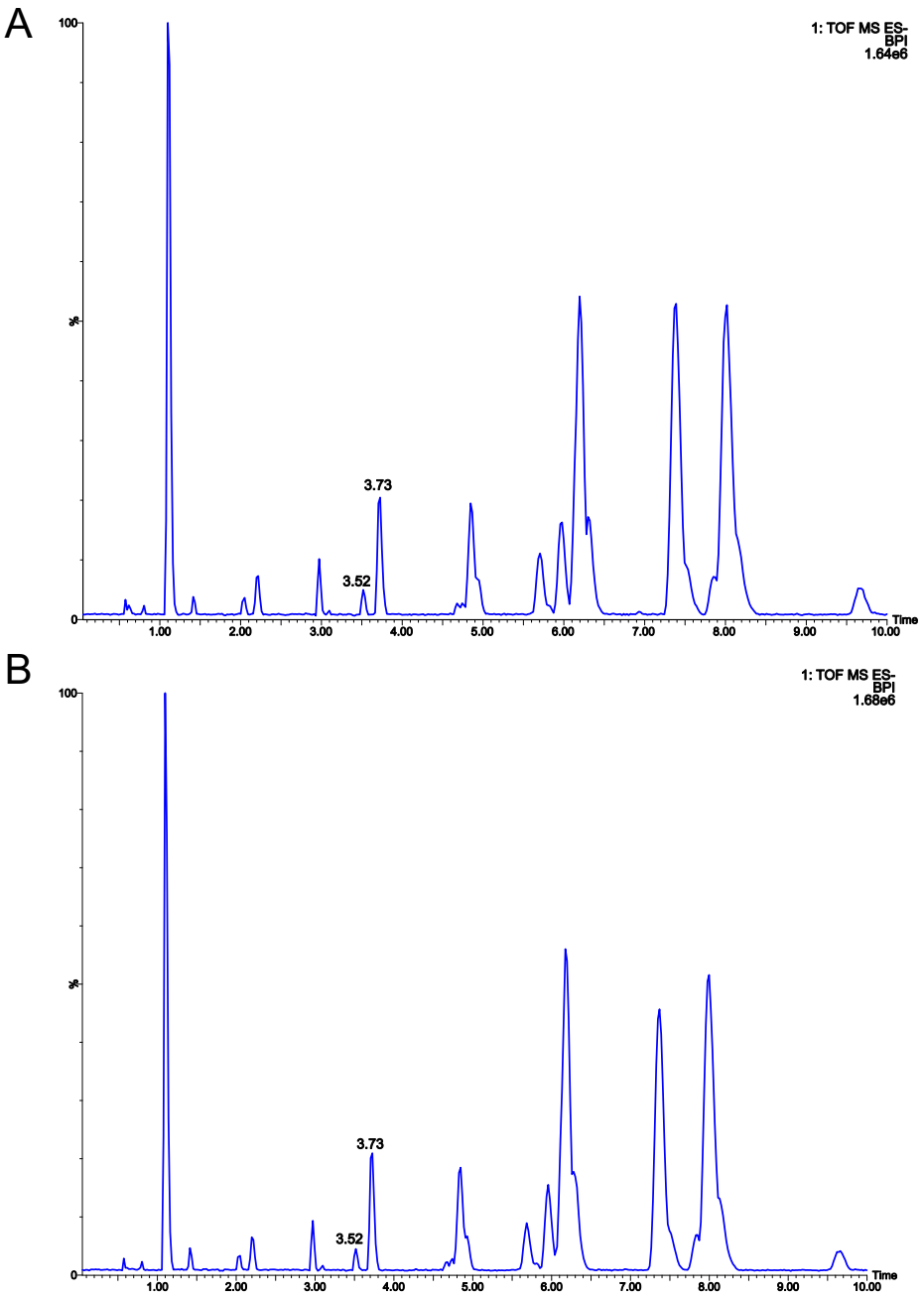


Figure S26. The lipidome of cells treated with 3 does not differ from that of untreated cells. LC trace of intact lipids extracted from cells (A) untreated with azide fatty acids, or (B) treated with **3**. The profile of (B) does not differ significantly from the LC trace of untreated cells in (A), suggesting that **3** is not incorporated into cellular lipids.

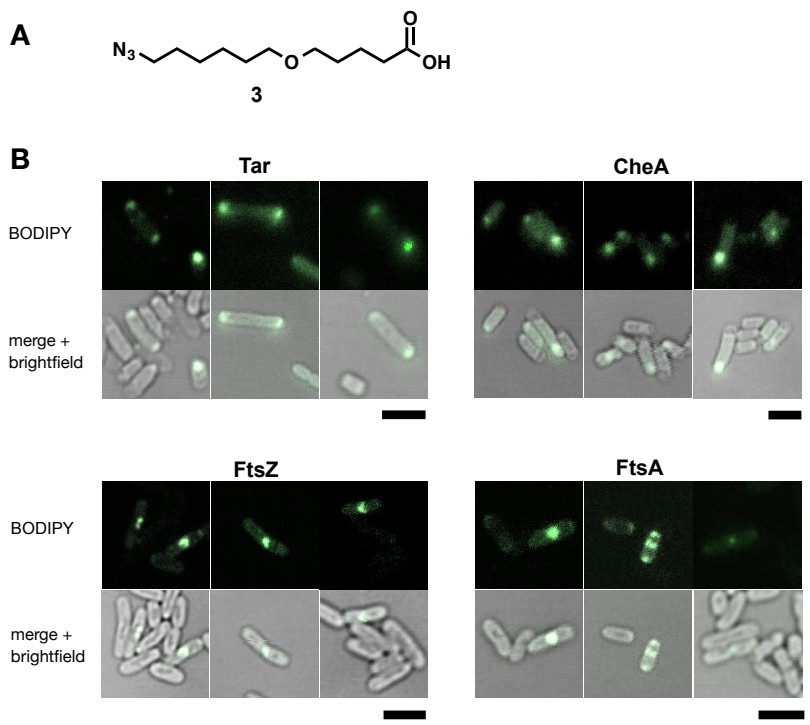


Figure S27. Representative live-cell images for chemotaxis and cell division proteins labeled with 2 and 3. (A) Structure of fatty acid analogue **3** used for live-cell labeling. (B) *N*-terminal fluorescence labeling shows polar localization for Tar and CheA and septal localization for FtsZ and FtsA (scale bar = 1 μm).

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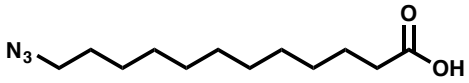
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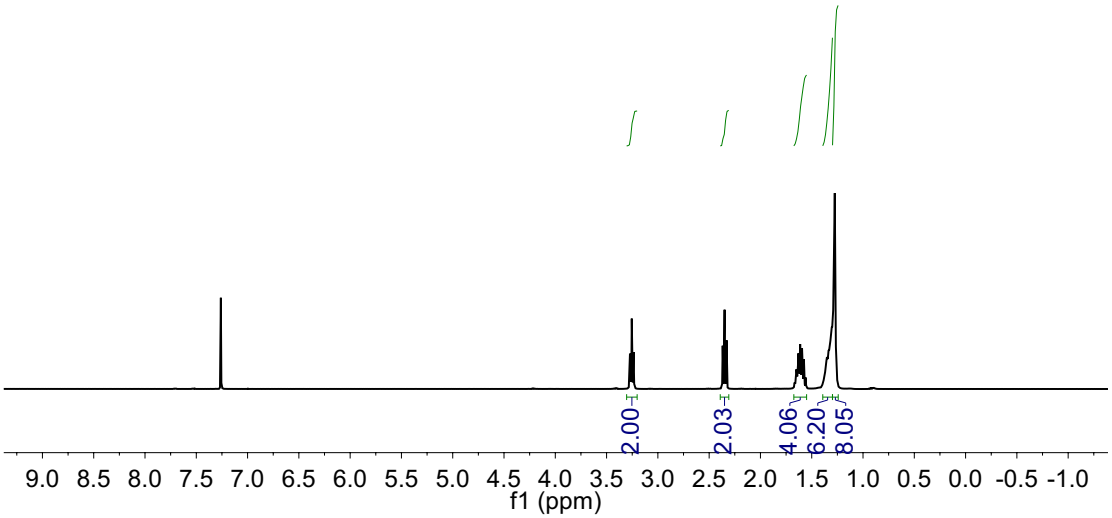
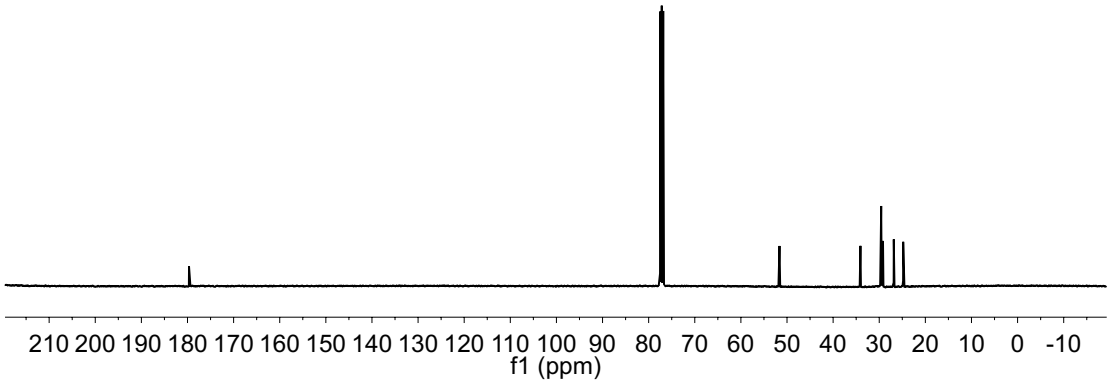
N3
OH


2.00
2.03
4.06
6.20
8.05
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)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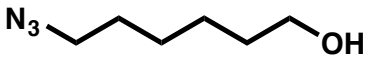
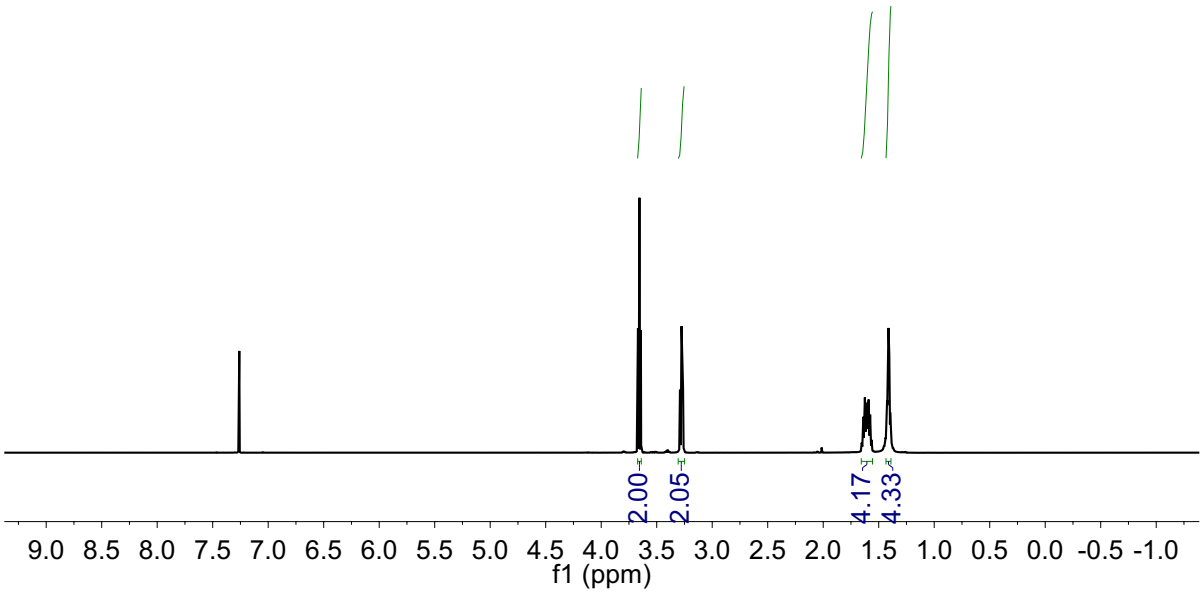
f1 (ppm)


N3
OH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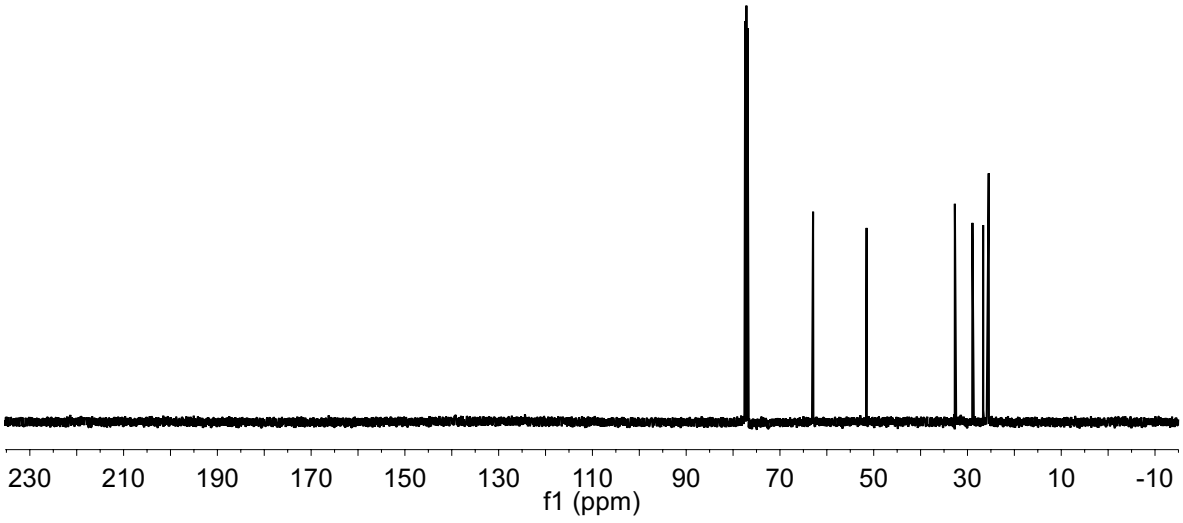
2.05
4.17
4.33
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)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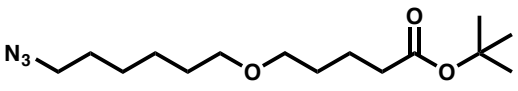
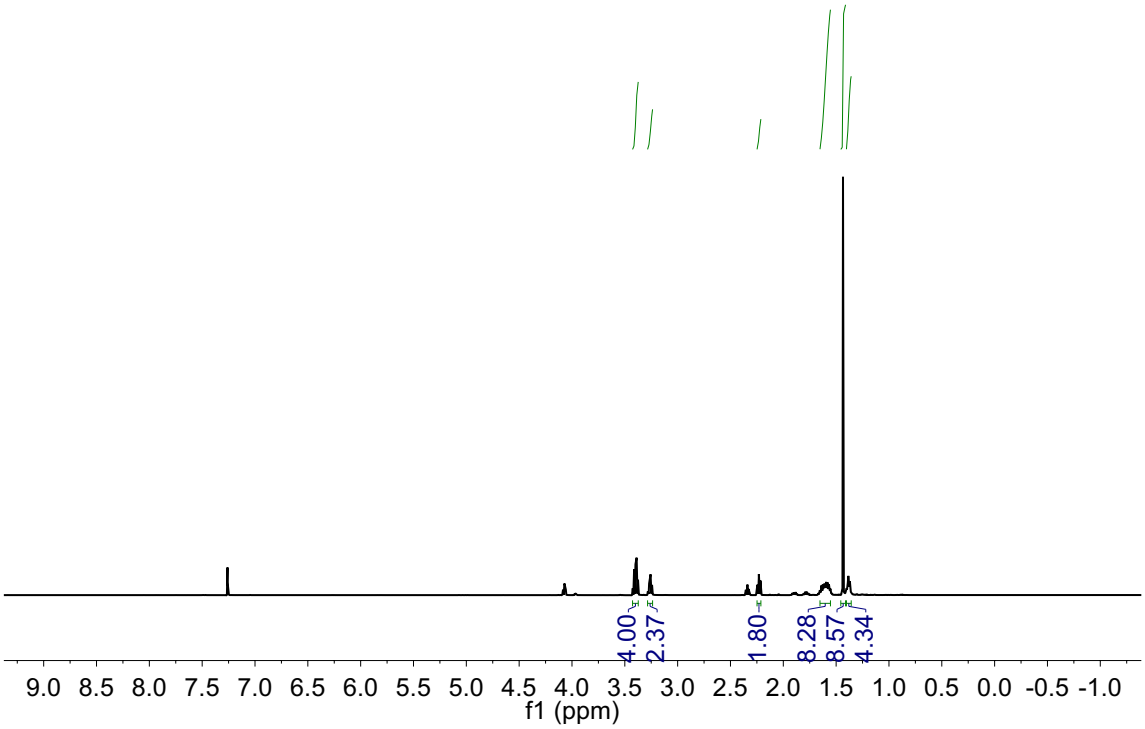
f1 (ppm)


N3
O
O
O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1.80
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8.57
4.34
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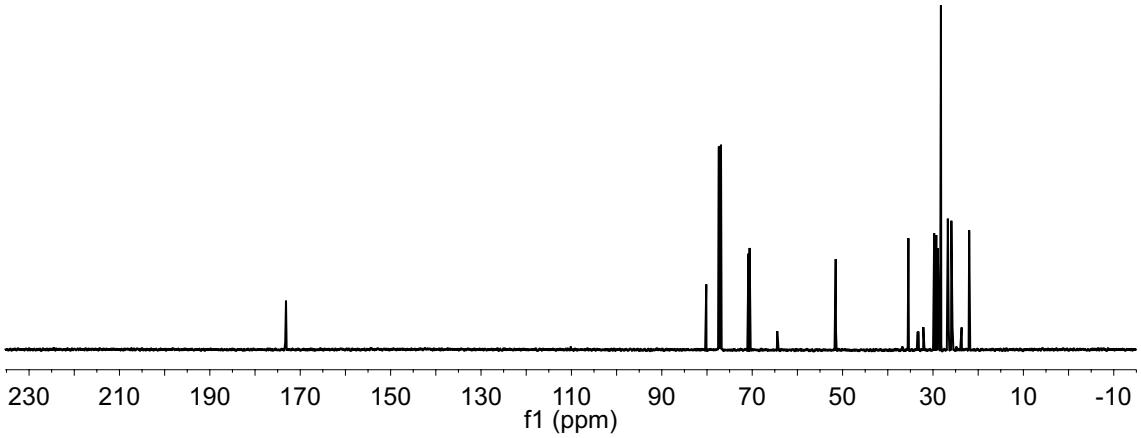
f1 (ppm)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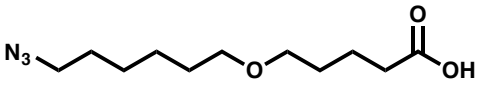
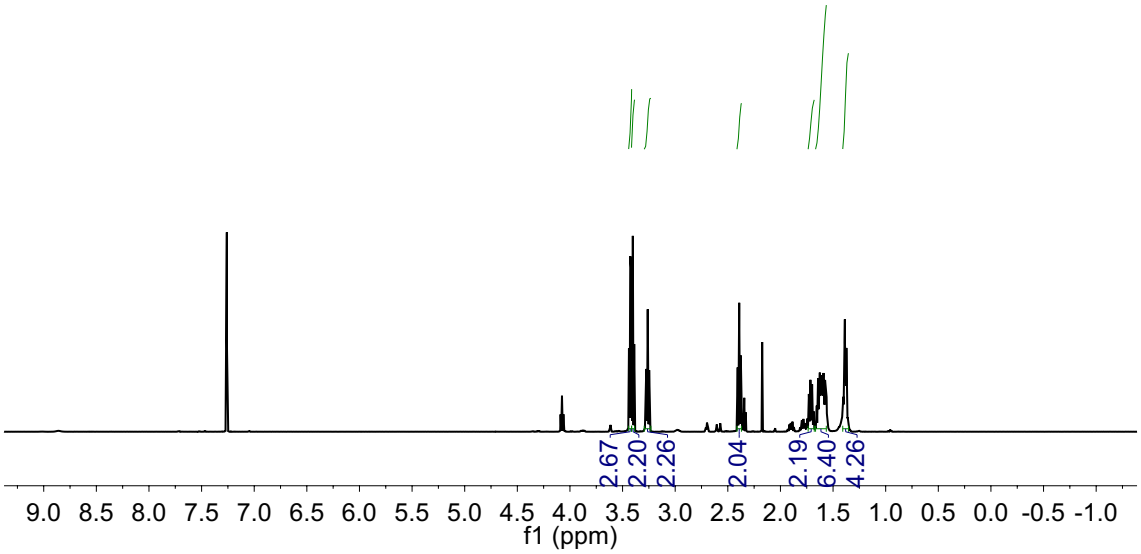
f1 (ppm)


N3
O
O
OH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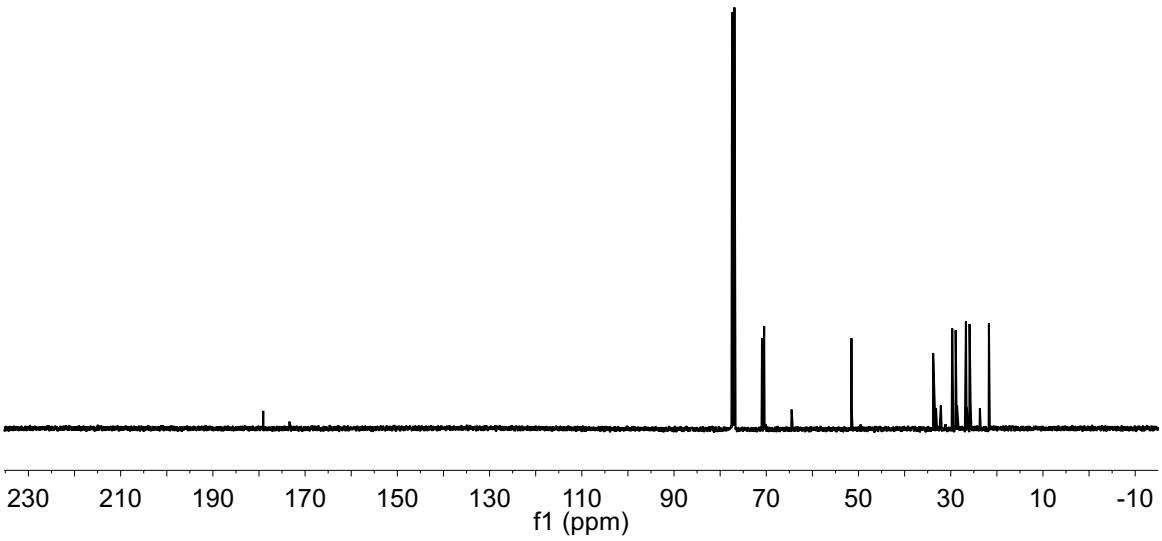
2.26
2.04
2.19
6.40
4.26
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)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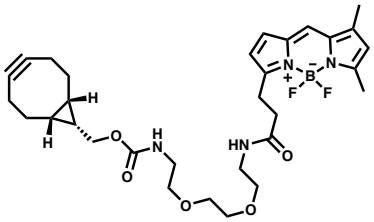
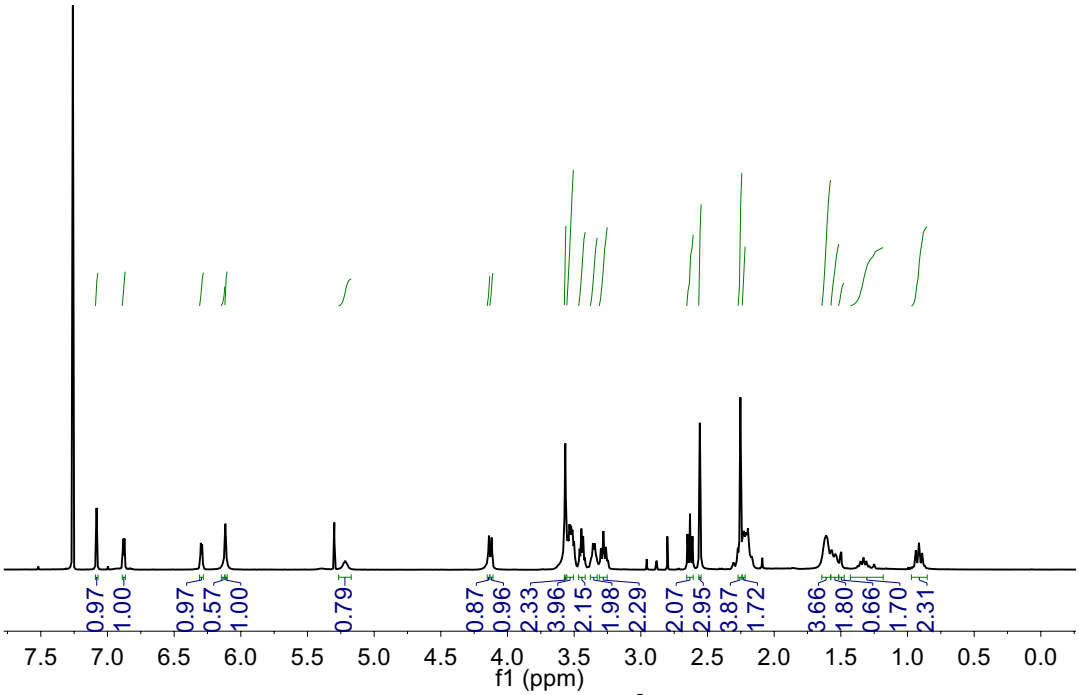
f1 (ppm)

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0.79
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0.96
2.33
3.96
2.15
1.98
2.29
2.07
2.95
3.87
1.72
3.66
1.80
0.66
1.70
2.31
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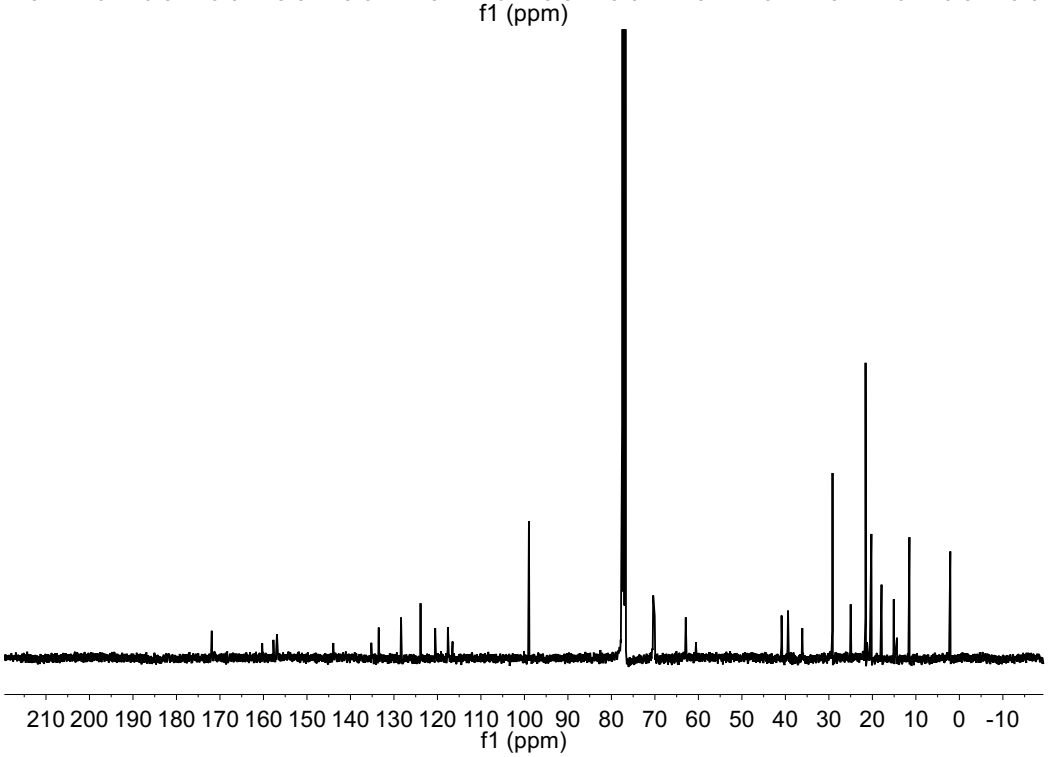
f1 (ppm)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